EFFECTS OF ATROPINE SULPHATE ON HIPPOCAMPAL RHYTHMIC SLOW ACTIVITY DURING A CONDITIONED LOCOMOTOR RESPONSE IN THE CAT

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Abstract. In cats atropine sulphate injected intraperitoneally in doses of 1.0 and 2.0 mg/kg abolished the hippocampal rhythmic slow activity (RSA) accompanying the performance of instrumental active avoidance response in a shuttle box. The action of atropine consisted in a gradual elimination of RSA, the RSA of slower frequency occurring during latency (the period of attentive immobility between the CS onset and the beginning of the conditioned locomotor response) and during the poststimulus period disappeared first, and that of a higher frequency, accompanying the initiation of the conditioned locomotor response and barrier crossing disappeared last. The speed of this process was dose dependent. The data show that the whole spectrum of RSA in the cat is atropine sensitive, but the level of this sensitivity depends on the RSA frequency.

INTRODUCTION

A very characteristic feature of the hippocampus is its ability to generate a high amplitude rhythmic slow activity (RSA or theta rhythm) during certain behavioral states (1, 2, 9, 19, 22). Recent investigations on rodents suggest that there are two forms of RSA. Each form has different behavioral correlates and depends on the activity of a separate neuronal system (10, 12, 13, 15, 19, 20). According to these data, a low
frequency form of RSA (4-7 cps in the rat) accompanies states of arousal or attention without overt motor behavior. Its appearance is blocked by intraperitoneal injections of atropine sulphate what suggests that it is mediated by a cholinergic (muscarinic) system. The second, high frequency form of RSA (7-12 cps in the rat) accompanies movements termed as “voluntary” (19). This form survives anticholinergic treatment, what suggests that it depends on the activity of another neurotransmitter system. It is not certain at present whether a similar duality exists in other animal species. Several experiments on cats have shown that in this animal intraperitoneal injections of atropine eliminate RSA. Some of these data however, were obtained on immobilized animals (18), or in conditions in which the locomotor activity was seriously restricted and long sections of the EEG activity were analysed mathematically (3). In freely moving cats Bennett (2) has found that intraperitoneal or intraseptal injections of another muscarinic blocker — scopolamine — eliminate completely RSA during the performance of an instrumental, alimentary response. However, as the author has concluded, the appearance of RSA in his experiments was not correlated with movements. In our recent experiments on cats (6) we have found, contrary to some literature data (7), that RSA accompanies the performance of a well stabilized instrumental active avoidance response (AAR) in a shuttle box, showing similar changes of frequency (although in a narrower range) as it was observed in the rat (21). Therefore we became interested whether the RSA of a higher frequency (5.5–6.5 cps) recorded during the conditioned locomotor response would show the same sensitivity to atropine as the RSA of a lower frequency (3.5–5.5 cps) observed during latency and after the response performance.

MATERIAL AND METHODS

The experiments were performed on four adult male cats with bipolar electrodes (1.0 mm tip separation) implanted into the dorsal or posterior part of the hippocampus. Before the experiments with atropine all cats were taught to avoid an electric footshock (1.0–1.5 mA) in a shuttle box by crossing a 10 cm high barrier within 10 s after the CS (a pure tone of 1,000 Hz) onset. The start of the CS was operated manually. The weight of the cat’s body on the pivoted floor after crossing activated microswitches which turned off the CS and US simultaneously. The intertrial interval varied from 20 to 40 s. The surgery and the training procedure were described in details previously (6). Hippocampal EEG,
animal's movements and the CS onsets and offsets were recorded on an 8-channel electroencephalograph (Medicor, Budapest). Changes in the hippocampal EEG were tested after intraperitoneal injections of two doses of atropine sulphate (BDH, London): 1.0 and 2.0 mg/kg. A 0.9% NaCl solution served as solvent for the crystalline atropine salt and as the fluid for control injections. The injected volume was 3.0 ml in all cases. The procedure was as follows. After putting the cat into the shuttle box hippocampal EEG was recorded for a minute or two without application of CS or US. Then ten trials were run. They were followed by a "struggle test". It consisted in holding the cat by its hindlegs for several seconds in such a way that only its forelegs were touching the floor. In order to wrench itself free, the animal struggled vigorously. As we noted before, RSA was almost continously present in the hippocampus during such a test. Afterwards, the cat was removed from the shuttle box and allowed 10 min rest outside. After the rest it was given an intraperitoneal injection of the drug or NaCl alone. Immediately after the injection the procedure was repeated i.e., ten trials were run followed by the struggle test and several minutes of rest outside. Successive repetitions of the procedure (denoted as "test periods") were performed at 15 min intervals between the starts of successive blocks of trials. The whole testing was composed of six test periods denoted as: 0 — before the injection, 1 — immediately after the injection, and 2, 3, 4 and 5 — at 15 min intervals after the injection. Each animal was tested twice under each of the three conditions: injection of 0.9% NaCl, atropine in a dose of 1.0 mg/kg and atropine in a dose of 2.0 mg/kg. The injections were made in a random order at intervals of no less than three days. All hippocampal EEG records in this experiment were done monopolarly at 1–35 Hz filter settings. The analysis of the data consisted in determining whether RSA was or was not present during the following periods (denoted as "trial periods"): latency — a period of attentive immobility between the start of the CS and the beginning of the locomotor response, performance — the time measured from the beginning of the locomotion to the CS offset, poststimulus period — 2 s following the CS offset. A section of activity was regarded as RSA when it consisted of regular waves (at least three in succession) of 3.5–7.0 cps frequency. When at least one such section was found during a given trial period, it was scored as "RSA present" without regard whether it was present during the whole period or during a part of it only. After the end of the experiment the cats were sacrificed and their brains were subjected to a standard histological procedure for verification of the electrode placements.
RESULTS

The location of electrodes from which the analysed records have been obtained are presented in Fig. 1. A detailed analysis of the changes in the cat's hippocampal EEG during AAR in the same experimental situation has been published earlier (6). In all cats tested in the present experiment without drug a clear cut RSA of 5.5–6.5 cps (mean 5.8 cps) frequency was present during the response performance. In the majority of trials RSA was also present during latency and during the poststimulus period, although its frequency was lower during these trial periods and ranged from 4.0 to 5.5 cps (mean 4.7 cps) during latency and from 3.5 to 5.0 cps (mean 4.3 cps) during the poststimulus period. Changes in the hippocampal EEG during spontaneous crossings (intertrial responses) were similar to those observed during the CS elicited responses. Injections of atropine resulted in an increase of locomotor activity. The responding to the CS was correct in majority of trials, although the US application was sometimes necessary in further test periods. This, ho-
wever, happened at a similar frequency after NaCl as well as after atropine injections. The changes in the hippocampal EEG consisted in an increase of amplitude and of the number of slow, irregular waves during intertrial intervals and in a gradual elimination of RSA from

![EEG records after atropine injections.](image)

**Fig. 2.** Fragments of records obtained during trials at different times (denoted to the left) after injection of 2.0 mg/kg atropine sulphate. HD, dorsal hippocampus; M, record from the movement sensor activated during locomotion. Arrows directed upwards and downwards denote the CS onsets and offsets, respectively. The horizontal dashes under the EEG records denote sections of activity regarded as RSA.

the record during trials. It was replaced by a mixture of small and large irregular waves, with the small component dominating at early test periods after injections and the large one at the end of testing (Fig. 2). In order to analyse the process of RSA elimination we computed the percentage of trials during which RSA was present. Separate computations were performed for each trial period during each test period. Then, the data were transformed into arcsin\(\sqrt{P}\) and a
factorial analysis of variance (Treatment × Trial Period × Test Period × Subjects) was performed. The analysis revealed a strong effect of Treatment ($F_{2,6} = 116.50, P < 0.001$) developing gradually in time, which was substantiated by a significant effect of Test Period ($F_{5,15} = 37.47, P < 0.001$) and a significant Treatment × Test Period interaction ($F_{10,30} = 22.96, P < 0.001$). A significant effect of Trial Period ($F_{2,6} = 5.17, P < 0.05$) and a significant Treatment × Trial Period interaction ($F_{4,12} = 7.81, P < 0.005$) suggested that the effect of injections was not equally expressed in all trial periods. This latter difference depended on the time elapsing from the injection, which was confirmed by a significant Trial Period × Test Period interaction ($F_{10,30} = 4.62, P < 0.001$) and a significant Treatment × Trial Period × Test Period interaction ($F_{20,60} = 7.78, P < 0.001$). Detailed comparisons (Duncan's test) revealed no differences between successive test periods when 0.9% NaCl alone was injected. RSA was present during response performance in all trials and it was also present almost in all trials during latency and during the poststimulus period. There were also no differences between the test periods 0 and 1 when the results from all injection conditions were compared. A rapid decrease in the RSA occurrence started from the test period 2 after injections of both doses of atropine. In test periods 3, 4 and 5 RSA did not appear during latency and during the poststimulus period ($P < 0.01$ in comparison with test periods 0 and 1 and in comparison with the corresponding test periods after injections of NaCl alone). There were no differences between the effects of both doses of atropine on RSA during latency and during the poststimulus period. Contrary to the above, RSA during performance persisted for a much longer time after injections of atropine (see Fig. 2). After the 1.0 mg/kg dose, in test periods 3, 4 and 5, RSA during response performance appeared significantly less frequently than in the corresponding test periods after injections of NaCl alone ($P < 0.01$ in all comparisons) but significantly more frequently than in the same test periods during latency and during the poststimulus period ($P < 0.01$ in all cases) and significantly more frequently than during response performance after injection of 2.0 mg/kg atropine ($P < 0.05$ in all cases). In the latter conditions, in test periods 3, 4 and 5 RSA during response performance was almost totally absent. It appears then that the effect of atropine on RSA during response performance was dose dependent (Fig. 3). It is necessary to mention here that the presence of RSA during response performance after injections of atropine does not mean that RSA accompanied the whole motor act. In fact, when it appeared in further test periods, it was present only during the most dynamic phase of the conditioned locomotor response (during its initiation or during the very
crossing). Spontaneous crossings and exploration stopped being accompanied by RSA very soon after injections of atropine. It was especially striking when the hippocampal EEG recorded during long periods of locomotion after atropine injections was compared with that recorded during similar, but short lasting behavior, after injections of NaCl alone. Whereas in the latter cases RSA was almost continuously present during the locomotion, in the former one a large, irregular activity dominated (see Fig. 2).

<table>
<thead>
<tr>
<th>Test periods (in min)</th>
<th>Injection</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td></td>
<td>control</td>
<td>0-5</td>
<td>15-20</td>
<td>30-35</td>
<td>45-50</td>
<td>60-65</td>
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<tr>
<td>0.9% NaCl</td>
<td>±0.4</td>
<td>±0.6</td>
<td>±0.6</td>
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<tr>
<td>Atropine</td>
<td>±0.6</td>
<td>±0.6</td>
<td>±0.5</td>
<td>±0.7</td>
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<tr>
<td>1.0 mg/kg</td>
<td>±0.6</td>
<td>±0.4</td>
<td>±0.6</td>
<td>±0.5</td>
<td>±0.7</td>
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<tr>
<td>Atropine</td>
<td>±0.6</td>
<td>±0.4</td>
<td>±0.2</td>
<td>±0.2</td>
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<tr>
<td>2.0 mg/kg</td>
<td>±0.6</td>
<td>±0.4</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.3</td>
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Fig. 3. Effects of atropine on the RSA occurrence during successive test periods. Ordinate, RSA occurrence expressed as the percentage of the number of trials performed \( \frac{\text{number of trials with RSA present}}{\text{total number of trials}} \times 100 \); abscissa, test periods in succession; dashed lines, RSA occurrence during latency; solid lines, RSA occurrence during response performance; dotted lines, RSA occurrence during the poststimulus period.

TABLE I

Mean RSA frequency (in Hz) during the AAR performance after injections. Asterisks denote mean values of the RSA frequency in cat no. 480 in which RSA was present in some trials till the end of the test.
Atropine did not seem to influence the RSA frequency. When RSA was present during a particular trial period, its frequency was in the same range as that characteristic for the same period before the injection. Since after injections of atropine RSA disappeared very soon during latency and during the poststimulus period, a rough estimation was made for the response performance only (Table I). RSA amplitude varied greatly during trial periods (see also ref. 6). Therefore any conclusive statement concerning this measure cannot be made on the basis of the present study.

During the "struggle test" RSA of 5.0–6.5 cps (mean 5.6 cps) frequency dominated the hippocampal EEG before the injections of atropine. 45 min after the injections only a mixture of slow and fast activities was recorded during this test (Fig. 4). The effect was similar after the use of both doses of atropine.

![Fig. 4. Records of the hippocampal EEG activity during the “struggle test”. A, before the injection of atropine; B, 36 min after the injection of 2.0 mg/kg atropine. HD (4-7 Hz), record from the θ (theta) channel of the EEG analyser; HD (1-35 Hz), original EEG record from the dorsal hippocampus; M, record from the movement sensor. Arrows directed upwards and downwards denote the start and the end of the test, respectively.](image-url)
DISCUSSION

The experiments presented above have confirmed earlier observations showing that in cats intraperitoneal injections of atropine abolish the generation of RSA in the hippocampus (2, 18). The new discovery of these studies is the finding that the process of RSA disappearance after atropine injections follows a certain order. As we have seen, the slower frequencies of RSA accompanying the attentive immobility during latency and the postural adjustments after the response performance, disappeared first and the highest frequencies, recorded at the beginning of the conditioned locomotor response, disappeared last. Since it is reasonable to assume that the time course of this process was dependent on the gradual increase of concentration of atropine in the central nervous system, one may conclude that RSA of a higher frequency is less sensitive to systemically applied atropine than RSA of a lower frequency. Nevertheless it seems to be certain that the whole RSA spectrum in the cat may be finally eliminated by atropine, which suggests that in this species the same RSA generating system operates during movements as during immobility. The fact that in the cat it is difficult to distinguish immobility related and movement related forms of RSA on the basis of frequency — RSA frequency during attentive immobility (latency) might be higher than that during postural adjustments after the response performance — speaks in favour of the above statement. There are some data which show that also in rodents the high frequency (above 7.0 cps) movement related RSA may be eliminated by intraperitoneal injections of scopolamine in a dose of 10 mg/kg (17). Also atropine in a dose of 25 mg/kg may reduces the appearance of RSA during movement in young rats (5). These data allow one to assume that the preservation of the high frequency RSA during movements, which was observed in many experiments (13, 19) might be due to an incomplete blockade of a single system. It might be added that, in spite of several attempts, the second hypothetical (noncholinergic) RSA generating system has not been identified anatomically nor pharmacologically (10, 12, 13). A stronger support for the existence of two RSA generating systems has been provided by data which showed that in the rat intraventricular injections of hemicholinium-3, a drug which depletes brain acetylcholine, do not prevent the appearance of RSA during movements but eliminate RSA during immobility (14). However the authors did not measure the level of acetylcholine depletion in their experiments. In the cat intrahypothalamic injections of hemicholinium-3 seem to eliminate RSA completely (4).
According to some data, RSA frequency may be regarded as an indicator of the density of impulsation reaching the hippocampus from unspecific brain stem areas via the septal pacemaker (1, 11). If the above is correct, then the effect of atropine observed in our experiments might be due to a gradual rise in the excitability threshold of the cholinceptive hippocampal neurones involved in RSA generation. Therefore after injections of atropine, RSA might appear only when the density of incoming impulses was sufficiently high to overcome the blockade of the hippocampal muscarinic receptors. It is well known, however, that other brain areas are also sensitive to muscarinic agents and blockers (8). Some authors pointed to the posterior part of the hypothalamus (18) or septum (2) as the crucial place of the RSA blocking action of atropine. On the other hand, Whishaw et al. (20) found that intraventricular administration of atropine sulphate did not abolish the immobility-related RSA in the rat. This finding makes the whole problem very complicated. Thus, the crucial site of RSA blocking action of atropine remains to be demonstrated.

REFERENCES


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