MESCALINE CONVULSIVE SPIKES TRIGGERED BY DIRECT CORTICAL STIMULATION [1, 2]

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INTRODUCTION

Mescaline has dramatic psychotropic effects in humans, causing alterations of color sensation and visual hallucinations. Mescaline also produces auditory alterations but no involvement of somesthetic sensation (Huxley 1954; Cholden 1956). Such specialization suggests the possibility that mescaline may have specific effects in the visual and auditory sensory areas of the cortex. The direct cortical response (DCR) has been used in this laboratory to investigate the effect of pharmacological agents topically applied to the cortex (Ochs and Hunt 1960; Ochs *et al.* 1960). The same approach was therefore used to investigate a possible differential effect of mescaline on the DCR excited in visual and other cortical areas.

Rovetta (1956) has previously studied topical application of mescaline. Using the relatively high concentrations of 20–33 per cent, he reported that it was a convulsant agent. Our studies soon revealed that mescaline was a convulsant agent though effective at a much lower concentration. The properties of mescaline spikes were studied and in particular of those spikes driven by an electrical stimulation. Our special interest was in a possible causal relationship of the mescaline spike to the DCR preceding it.

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METHODS

Forty-two rabbits and five cats were examined. Their cortices were exposed after the animals were anesthetized with ether. For the phenomena to be observed it was found essential to examine the curarized preparation. Flaxedil was given and a Harvard respiration pump used. Local anesthesia of the cut surfaces with procaine was also used and in some cases not, so that the results to be described are not to be attributed to the effects of procaine. Pentobarbital was injected intravenously in some experiments as noted in the Results.

Bipolar stimulating electrodes made of fine insulated wire closely spaced with tips bared were applied to the surface of the cortex. The separation of the tips was 20–50 μ. Brief 10 μsec stimulating pulses were applied and repeated at 3 sec intervals. Little evidence was noted of progressive changes of the DCR even after hours of such excitation (Ochs and Booker 1961). Further details of the technique of stimulation and recording of the DCR may be found in that paper. DCRs of the usual form and amplitude were recorded with a monopolar method. The active electrode was placed approximately 2 mm from the stimulating pair; the indifferent electrode was threaded through neck muscle or placed inside of the reflected skin of the scalp. A negative deflection is displayed in the figures as an upward movement of the oscilloscope beam. The responses were photographed with a Grass Kymograph camera and frames identified with an automatic timer-marking device.

To study the effects of mescaline a small drop of mescaline solution (usually 1% in Ringer solution) was placed on the cortex at the site of the recording electrode. The presence of this

additional fluid around the electrode produced only a small amount of the electrical shunting, as judged from the relatively small change in amplitude of the response (maximum reductions in amplitude of the DCRs did not exceed 15%). The cortex could not be excessively moist, otherwise the drop of fluid containing mescaline would flow away from the application site.

Where an island of cortex was to be prepared, a fine knife was used (Ochs 1956) with vertical blade portion of such a length that all neuronal connections including the underlying cortico-cortical fibers were cut leaving only the pial vessels of the intact pial surface to supply the island. Islands were approximately 6–8 mm square. When a chronic island was to be prepared, rabbits were anesthetized with pentobarbital and a trephine hole 1/2 in. in diameter was made in the skull. The end of the special knife used to make islands was poked down through the dura. As it was moved forward to make the cut its smooth upper part passed under the pia and dura. After the island was made the defect in the skull was covered with a plastic disc which was cemented with acrylic resin to small jeweler's screws set around the hole. In later experiments a threaded metal shell was screwed into the trephine hole in the skull and a short plastic cylinder set down inside it to the level of the brain surface and held in place with set screws. The skin was stitched over the plastic covering and the wound in turn covered with sealskin (Collodion preparation, Aloe). One ml of procaine-penicillin was injected i.m. Three to seven days later the animal was anesthetized with ether, the plug was removed, the skull defect widened, and the dura removed. Direct cortical responses were then elicited in the island as usual and the effects of mescaline studied. In most cases acute and chronic islands were checked histologically for completeness of the cuts.

RESULTS

Mescaline spike activity

Mescaline is effective in producing convulsive activity in both the intact cortex and in cortical island preparations. Its action can be seen in the acute island preparation from which negative wave DCRs were first obtained as control responses (*C* of Fig. 1). A small drop of a 1 per cent concentration of mescaline in Ringer fluid was then added to the recording site. During the next 4–5 min the negative wave DCR became progressively smaller in amplitude and longer in duration. That electrical shunting by the drop of mescaline solution was not the cause of the reduced DCR amplitude was shown when the fluid around the recording electrode was removed and the same diminished amplitude and broadened response remained. After 6 min a mescaline spike appeared. This gradually became larger

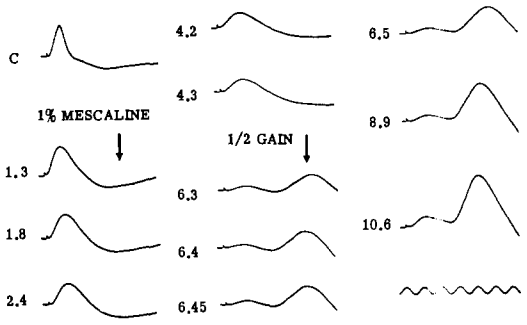

Fig. 1
Relation of mescaline spikes to DCRs. Control DCRs are recorded (*C*) and then a drop of Ringer's containing 1 per cent mescaline placed on the recording site. Numbers to left of frames refer to time in minutes thereafter. The DCR becomes smaller in amplitude and of longer duration. After 4.3 min the gain was halved (1/2 gain) and the mescaline spike appeared and then grew progressively larger. Acute island preparation. Rabbit, visual area. Calibration: sine wave 100 c/sec, 0.37 mV.

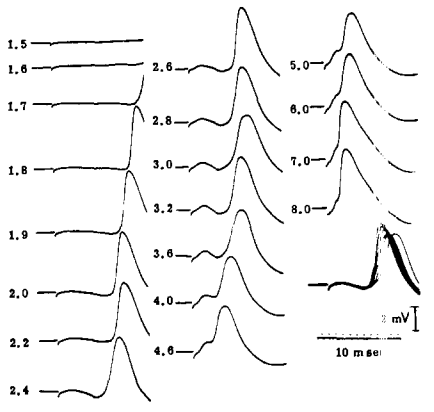

Fig. 2
Effect of stimulus strength on mescaline spike delay. The figures refer to strength of stimulus in arbitrary units. Seven traces are overlayed in the last trace in the lower right hand corner at a relative strength of 2.8. Rabbit. Acute island, visual area. Time 10 msec, amplitude 2 mV.

(6.3 to 10.6 of Fig. 1) until it reached several times the height of the DCR. The spike could attain amplitudes of 4–6 mV; its duration was approximately 30–60 msec.

The latency between the DCR and the spike could be longer than that pictured in Fig. 1 and depended in part upon the strength of stimulation. With weak stimulation the latency of the "driven" spike could be over 100 msec (Fig. 2). As stimulating strengths were gradually increased, the spike appeared earlier and earlier with respect to the stimulus. A DCR preceding the spike appeared at the increased stimulating strengths and by further increasing the strength of stimulation DCR and spike would merge. At any given stimulus strength the latency showed some fluctuation. This is indicated by the last tracing in Fig. 2 where seven responses at the arbitrary stimulus strength of 2.8 were overlaid and the mescaline responses are seen to vary in latency and in amplitude.

The two examples of Fig. 1 and 2 were from acute island preparations, where the delayed appearance of the mescaline spike was more readily observed than was the case in the intact cortex. In the intact cortex the spike tended to merge more readily with the preceding DCR (Fig. 3). It was not so easy to obtain the very long latencies between the stimulus and the driven spike. However, separations between the

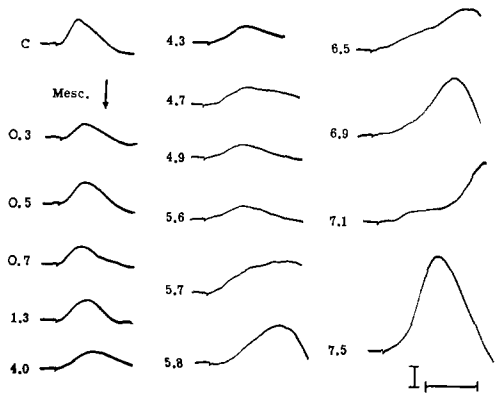

Fig. 3

Development of mescaline spike response. DCR control (*C*), then 1 per cent mescaline added to recording site. Time in minutes thereafter. Irregular appearance of mescaline spike and then a uniform mescaline response began at 7.5 min. Rabbit, visual area. Calibration: vertical bar 1 mV, horizontal line 30 msec.

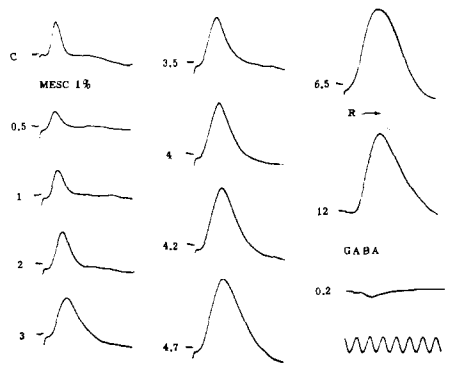

Fig. 4

Mescaline spike activity in chronic island preparation. Island prepared 6 days before examination. Control DCR at (*C*) and 1 per cent mescaline placed on the recording site. A mescaline spike is excited from the onset of the DCR at the time shown in minutes thereafter. Wash at (*R*) with Ringer solution made little difference in amplitude height. GABA placed on the cortex after the response at 12 min produced a rapid block of the response and a small reversed potential corresponding in time to the control DCR. Rabbit, visual area. Calibration: sine wave 100 c/sec, 0.37 mV.

DCR and the spike which are relatively long were seen in the intact cortex in the early phase of mescaline action before a stabilized large spike appears (starting at 7.5 min in Fig. 3). Later in the declining phase of mescaline action, the smooth apparently homogenous response breaks up again into a separate DCR and spike (*cf.* Fig. 6). Mescaline activity may last an hour or longer.

The delay of the mescaline spike in the acute island preparation cannot be ascribed to the island preparation *per se*. Fig. 4 is an example of spike activity taken from a chronic island preparation. In this case the spike appears to arise directly from a DCR. On the other hand, spikes which could have long latencies were also found in chronic island preparations. It should be noted that similar spike activity can appear in chronic island preparations several weeks old without adding mescaline. This kind of spike behavior can be readily distinguished from the mescaline spike activity obtained from chronic islands made 3–7 days beforehand.

The concentration of mescaline usually employed was 1 per cent and this has been found to be effective to produce spikes within 3–9 min. However, occasionally a second application of

mescaline has been required. Spike activity was also seen in a small number of trials after the application of a 0.5 per cent concentration of mescaline while a 0.25 per cent concentration was not effective in a few trials.

The usual effect of mescaline on the negative wave DCR was to depress its amplitude and prolong its duration. This was the first effect seen and occurred within a few minutes. The spikes, as already noted, took longer to develop.

The effect of mescaline on cortical regions other than the visual area was investigated. This drug applied to the frontal cortical region of the rabbit produced typical spikes and spikes were also produced in the suprasylvian gyrus of the cat. Application of mescaline also caused a repetitive discharge of spikes of the same waveform as the driven spikes but the former may appear for only a short time. These spontaneous spikes at times occurred just before a driven mescaline spike or DCR. In this event the subsequent potential would be greatly decreased in amplitude — much like an occluded response (see Discussion).

The effect of GABA on mescaline spike activity

Since gamma amino butyric acid (GABA) may block or invert strychnine and picrotoxin spikes (Jasper 1960) it was of interest to determine its effect on mescaline spikes. In the chronic

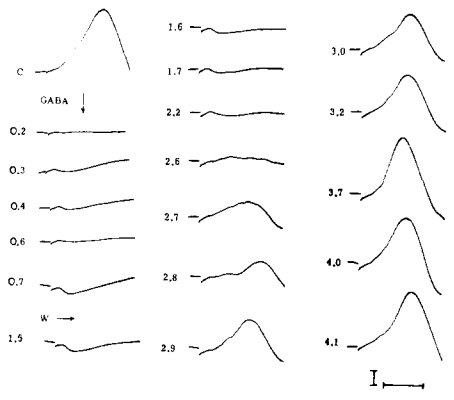

Fig. 5

Effect of GABA on mescaline spikes. Continuation of example of Fig. 3. Control mescaline spike (*C*) and 1 per cent GABA added to recording site. Time in minutes thereafter shown to left. Surface washed (*W*) with Ringer fluid after 0.7 min and mescaline spikes gradually return. Calibration as in Fig. 3.

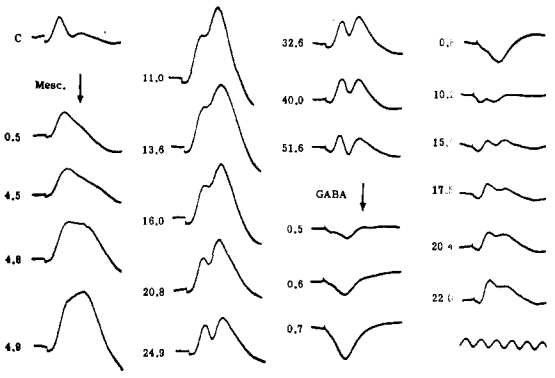

Fig. 6

Dissociation of mescaline spike and the effect of GABA. A control (*C*) DCR is shown and then 1 per cent mescaline added to the recording site. Times thereafter shown in minutes. After 51.6 min, 1 per cent GABA was added to the recording site and recovery thereafter. Rabbit, visual area. Calibration: sine wave 100 c/sec, 0.37 mV

island experiment shown in Fig. 4 when a large uniform spike was present, a small drop of 1 per cent GABA was applied to the recording site: within seconds, the spike was eliminated. A small potential of reversed sign remained which corresponded in time after the stimulus to the DCR. The blocking effect of GABA on mescaline spikes obtained from intact cortex is shown in Fig. 5. Here also a rapid effect can be seen with an inverted wave corresponding to the DCR (Control in Fig. 3). After washing with Ringer's solution, spike activity returned fairly rapidly.

Occasionally an irregular DCR pattern having a broader and often double curve may be found (Ochs and Booker 1962). In Fig. 6 the spike appears to arise as an augmentation of the second negative wave of such a DCR. Later in the course of mescaline activity the response appears more clearly separated into a DCR and the spike. An attempt was made to see if a separate inversion of the DCR and of the later spike would occur when GABA was placed on the recording site. A distinct inversion of each of these responses was not evident, although a small separate inversion was fleetingly suggested during recovery (at 10.2 min after GABA in Fig. 6). A larger inverted wave was also seen early in the course of GABA action (0.7 min after GABA in Fig. 6). Large inverted waves are sometimes seen when the DCR is inverted by GABA. The different actions of GABA on the

DCR and on the spike can be seen in Fig. 8. In *C* an overlay of five traces shows a DCR and later the somewhat irregular spikes appearing after a slow positive swing. After GABA was applied, the spike and the DCR were blocked within a few seconds (*D*) and then later a small inversion corresponding in time to the appearance of the DCR was seen (*E*). Later in the course of recovery from GABA, an apparent reversal of the mescaline spike can be seen (*F*, *G*). However, in this example, the spike before GABA application had a positive phase and GABA is apparently blocking the negative part of it leaving its positive part component unaffected (*H*). It should be noted that a positive-negative sequence for the mescaline spike is not usually seen, the commonest type being the smooth negative wave shown in the other figures.

Pentobarbital and mescaline spike activity

An injection of pentobarbital caused a reduction in the amplitude of the spike which may be temporary or prolonged depending on the amount injected. In Fig. 7, a first i.v. injection was followed by recovery within a few minutes. After a second injection the spike remained decreased. The response which remains is the DCR which is known to be less sensitive to

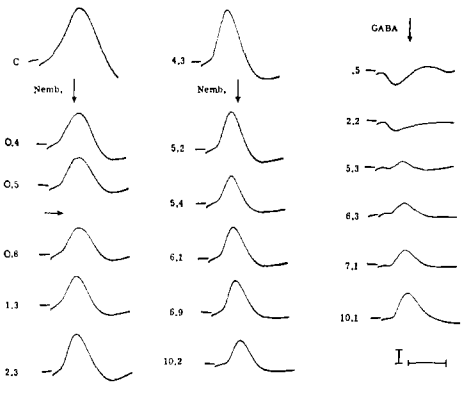

Fig. 7

Effect of pentobarbital on mescaline spike. Continuation of experiment shown in Fig. 3. After a control (*C*) mescaline spike, pentobarbital (*Nemb.*) was injected i.v. at a slow rate until at the arrow 30 mg was injected. 4.3 min later a similar 30 mg injection was given and the mescaline response fell in amplitude and a response similar to the original DCR control was seen (*cf.* Fig. 3). GABA placed on the recording site produced reversal and recovery as usual. Calibration as in Fig. 3.

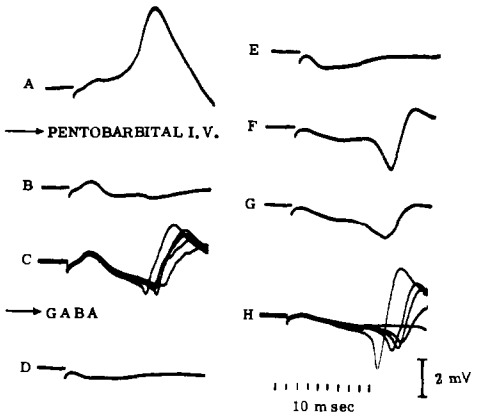

Fig. 8

Pentobarbital and GABA on a later appearing mescaline spike. *A*: control shows a DCR with a later larger mescaline spike (continuation experiment Fig. 2). *B*: 1 min after injection of 20 mg pentobarbital i.v. *C*: five overlayed traces after recovery and the 1 per cent GABA topically applied. *D*: 0.2 min, *E*: 1 min, *F*: 2 min, *G*: 2.1 min, and *H*: 5 min afterwards (five overlayed traces). Calibration as in Fig. 2.

pentobarbital (Ochs 1956). As an additional test to confirm its identity with the DCR, GABA was placed on the responding site and this resulted in the usual prompt block and reversal of the negative wave DCR with its eventual recovery. The selective elimination by pentobarbital of the mescaline spike can be better seen when this appears after the DCR with a rather long latency as shown in Fig. 8, *A*,*B*. Recovery occurred later (*C*) with the spike at this time somewhat irregular in form. Pentobarbital was effective in reducing spike activity in both the intact and in the island preparation. As would be expected from these results. spikes were not readily elicited by topical applications of mescaline when animals were anesthetized from the onset with pentobarbital; only a partial action of mescaline could be observed if the level of anesthesia was not too high.

DISCUSSION

The large spikes triggered by electrical stimulation after the topical application of a 1 per cent concentration of mescaline to the cortex are analogous to those produced by topical application of strychnine, *d*-tubocurarine, picrotoxin and other convulsants (Chang 1953; Jasper 1960). Mescaline, like other convulsant agents, can

also excite spike activity in the isolated island preparation (Rech and Domino 1960).

Our results confirm Rovetta's (1956) except that in the present experiments a much lower concentration of mescaline was found effective. Possibly the use of penetrating recording electrodes in Rovetta's study caused the cortex to be less sensitive to mescaline action. In further confirmation of Rovetta's findings, the effect was found not only in the visual area but also in forward areas of the rabbit brain and in the suprasylvian gyrus of the cat. The present techniques of study are insufficient to account for the subjective effects of mescaline related to the visual and auditory senses.

Triggered mescaline spike activity may appear to arise smoothly from the DCR or, in other cases, to occur after a relatively long latency. In the latter cases the latency of the spike could be controlled by varying the stimulus strength. This was not possible in cases where it arose with a small latency following threshold stimuli. Discharges of longer latency were found more often in the acute cortical island preparation, and possibly the presence of a short or a long latency type of driven spikes is related to the degree of intracortical excitability.

A relationship was reported (White *et al.* 1960) between the amplitude of the DCR appearing right after the stimulus and spike waves produced with pentylenetetrazol (metrazol) and with the injection of aluminum oxide (alumina cream). Both an early increase and a late decrease of DCR amplitude is seen in Chang's (1951) records of strychnine activity. In our studies the DCR amplitude was not augmented as mescaline spike activity developed. The DCR preceding the spike was only occasionally increased, but nearly always was decreased, in amplitude and this was the first indication of mescaline action. A similar decrease of the DCR can be seen after topical application of strychnine and pictrotoxin in Jasper's (1960) records. The decrease in DCR amplitude suggests that mescaline depresses the excitability of the apical dendrites to some degree. This depression presumably is overcome to some extent, as will appear from the following discussion.

With reduction of stimulus strengths, spikes are "driven" with long delays and there might be little evidence of the presence of a DCR (Fig. 2). The DCR changes in amplitude with stimulus strength while the spike appears "all-or-none", further suggesting the independence of the two phenomena.

The long latency between the stimulus and the driven spike is an interesting phenomenon. To account for such latencies we may consider theories of circulation of activity in looped chains of neurons (Burns 1958), a maintained membrane change, or a long-lasting repetitive discharge from "pacemaker" units (Ward 1961). In any case this unknown delay mechanism will eventually excite a sensitized group of intracortical neurons — the "epileptic" neuronal aggregate discussed by Ajmone Marsan (1961). This group appears to discharge in "all-or-none" fashion when threshold is reached and in turn (via synapses on apical dendrites — *vide infra*) to give rise to the surface discharge.

What intracortical elements are sensitized to give rise to the spike discharge remains to be determined. These neurons appear to be cells deeper in the cortex, as the time taken for the spike to develop would suggest. The special sensitivity of these deeper cortical cells is shown by their susceptibility to intravenously injected pentobarbital; this drug is acting at the cortical level because it can block spikes obtained from an "island preparation".

We can at least eliminate the possibility that mescaline only acts upon the terminals of specific afferents, since spikes were produced in cortical areas outside the distribution of primary sensory terminations. Mescaline was also effective in the chronic island preparations of up to 7 days, where the coarser fibers identified in silver-stained preparations as specific afferent terminations appeared to be degenerated after 2–3 days.

The discharge of the sensitized intracortical neurons appears to activate the apical dendrites they synapse on. The rapid block of the spike found with topical application of GABA is evidence for this concept. This is so if the spike is not secondarily excited by DCR activity as has been indicated. The rapid effect of GABA on the spike indicates that this is generated in the apical dendrites because little time would be needed for diffusion, an explanation used for the similarly rapid blocks of the DCR by GABA.

Mescaline spike discharges which occasionally appear spontaneously before a stimulus will cause an occlusive-like decrease in the subsequent DCR or mescaline response. The DCRs will show a similar decrease of the response to a second stimulus when temporally or spatiotemporally interacted (Ochs and Booker 1962), a behavior taken to indicate that the negative wave DCR is most likely a non-summating type of response in the apical dendrites (Ochs 1962). The occlusion of the DCR by the spike therefore suggests common neural elements, *i.e.*, that the spike represents activity of the apical dendrites. This, however, needs much more evidence to be fully accepted. For example, GABA did not cause a large inversion of the mescaline spike as it did for the DCR, although large inversions after GABA were reported with other convulsive agents under certain conditions (Jasper 1960). The differences between a DCR and mescaline spike may only be quantitative, the mescaline spike being a larger response of the apical dendrites compared to a more limited discharge produced during a DCR.

SUMMARY

1. Mescaline topically applied to the cerebral cortex in a 1 per cent concentration elicits paroxysmal spikes of up to 6 mV in amplitude and 30–60 msec in duration which can be driven by a brief electrical stimulation applied to the nearby cortex. Driven spikes were found in a number of cortical regions — including frontal regions of the rabbit and suprasylvian gyrus of the cat, in addition to the visual cortex of the rabbit. Spikes could be elicited in the acute and in the chronic cortical island preparation. The latency of the spike may be short and the response give the false appearance of an exaggerated direct cortical response (DCR) or the latency may be longer than 100 msec and varied by changing the stimulus strength.

2. The DCR is usually decreased in size soon after mescaline application and does not appear to have a causal relation to the spike. GABA rapidly blocks both the DCR and the spike, producing an inversion of the DCR but not of the spike.

3. The spike excited in the intact cortex or in an island preparation can be blocked by intravenous injections of pentobarbital while the DCR remains. A direct intracortical action of pentobarbital to depress the excitability of mescaline-sensitized intracortical neurons is thereby indicated. The sensitized neurons are believed to be deeper in the cortex and to synapse on pyramidal cell apical dendrites to generate the large convulsive wave in those membranes.

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