ONCE UPON A TIME: A RETROSPECTIVE OF NEUROENDOCRINOLOGY OF ANIMAL HYPNOSIS IN THE RABBIT

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ABSTRACT

In immobility by inversion and restriction (animal hypnosis), studied in Carli's lab in male rabbits in the late 1970s, the response to induction (the eliciting stimulus, regarded as an aversive event) was dissociated from response to hypnosis in terms of neuroendocrine systems activated and their time courses. Peripheral modifications of corticosterone (C) and testosterone (T), typical of the stress-response, were found following induction, not followed by immobility, with a marked and long-lasting increase in C (until 15 minutes after the end of the episode) and a parallel reduction of T. When induction was followed by immobility, there was a recovery of both hormones to control levels within 15 minutes. Hypnosis seems therefore to function as a sort of buffer – a compensation system re-establishing the previous endocrine balance with respect to a stressful event. This interpretation is supported by electrophysiological data, such as electroencephalography synchronization during hypnosis.

This recovery effect was present also in the adrenals, where the metabolites of the biosynthesis path of corticosteroids and of the adrenal androgens were modified in parallel to plasma alterations. In the anterior hypothalamus, hypnosis had no recovery effect on the depression of T metabolism, the latter being related to the neural circuits controlling sexual behaviour.

Key words: animal hypnosis, pituitary–adrenal system, pituitary–gonadal system, susceptibility, habituation

When I first met Giancarlo Carli, in the late 1970s, I was fascinated by his studies on animal hypnosis. Retrospectively, this was in fact the beginning of my interest in animal behaviour and its biological basis, which I developed later in my research.

Theoretical problems were raised by animal hypnosis at the time: in particular, whether or not the different immobility responses in various species—described with different names—refer to distinct phenomena or are the same phenomenon in different forms (the unitary explanation) (Lefebvre & Sabourin, 1977).

Experimentally, immobility by inversion and restriction of the animal was the most studied at various levels (e.g. stimulus, responses, physiological correlates); presented frequently at different evolutionary levels, it shows strong similarities in many species (homology). The model selected in Carli's lab was immobility by inversion and restriction in the male rabbit—a docile animal with a high susceptibility to this response and suitable for neurophysiological studies.

A relevant contribution was given by Carli's work on the neural control mechanisms (i.e. the localization of the neural centres necessary for the elicitation of the response) and the neurophysiological correlates of immobility response: in particular, the high voltage slow-wave pattern of electroencephalography (EEG) (synchronization) developed during the immobility response, alongside depression of reflexes and decreased muscle tone.

The subsequent step was to extend the study to the neuroendocrine correlates of animal hypnosis. These were the years of great development in the field of behavioural endocrinology, with fundamental findings in sexual differentiation and reproductive behaviour but also in the neuroendocrinology of responses to stress. The collaboration with Giancarlo Carli—and the beginning of a life-long friendship—was the result of the interests of a small group of people, including myself, to apply the rationale and methodologies of behavioural neuroendocrinology to animal hypnosis.

If the immobility response may functionally represent an extreme form of defence, the induction procedure—physical restraint in our case—can be regarded as an aversive, stressful event (Carli, 1982). Since the two components of induction and hypnosis are methodologically intertwined, our preliminary interest was to dissociate the response to induction (the eliciting stimulus) from hypnosis, in terms of neuroendocrine system activation and their time courses.

The question addressed was if and how hormones are influenced or influence this behaviour, by analyzing hormones (a) as correlates of susceptibility to immobility and (b) as modifications following immobility (effects).

The differences in susceptibility to hypnosis can be regarded as an example of the individual differences in the responses to environmental perturbations. This individual variability, hard to explain when interpreting experimental data, is in fact an opportunity to discover a variety of different mechanisms and strategies; this is the reason why studies of subsets of animals developed in various research fields, such as dominance/subordination, stress-responses, and anxiety profiles.

In animals, susceptibility to hypnosis is assessed through the mean duration of the episodes. Two subpopulations of animals can be distinguished: susceptible (SUS) and unsusceptible (UNS).

We selected two endocrine systems which are involved in adaptive responses and are sensitive to environmental stimuli, in particular to stress:

1. Hypothalamic–pituitary–adrenal axis: a system rapidly activated by stressors.
2. Hypothalamic–pituitary–gonad axis: with stress inhibiting the system.

In our research, we developed different protocols, according to the aim of study (see Table 1).

HYPNOSIS AND THE PITUITARY–ADRENAL SYSTEM

Corticosterone and susceptibility. Corticosterone is the final step in the hormonal cascade of the pituitary–adrenal system, the main glucocorticoid secreted by cortical adrenals in rodents (cortisol is secreted in humans).

Table 1. Protocols developed in the reported experiments

Protocol	Treatment	Criteria/Time Course	Hormones/Metabolites
(a)	5 trials/day 2 consecutive days	SUS: hypnosis duration > 45 sec	Plasma basal levels C *before* treatment
(b)	Single block, 4 trials/1 day matched groups: IND/HYP	Longitudinal effects: 0, 15, 30 min	Plasma C, ACTH, beta-EP: repeated measures *before/after* treatment adrenal metabolites
(c)	Single episode matched groups: IND/HYP	Effects after 15 min	Plasma T; T, hypothalamic metabolites
(d)	5 trials/day until habituation	Habituation: hypnosis duration < 45 sec, in 2 consecutive days	ACTH, T: plasma levels every day *before* treatment T hypothalamic metabolites

The correlation between corticosterone basal plasma levels (measured 24 hours before the treatment) and susceptibility to hypnosis has been proved in the experimental model of five trials per day for two consecutive days (Table 1(a)). Subjects were considered susceptible when the mean duration in the ten trials was above 30 seconds and the duration of the first episode was above 0 (with a positive correlation between the two criteria) (Carli et al., 1979).

Significant differences in basal C levels were found, with higher levels in SUS animals than in UNS, possibly indicative of a higher reactivity of the pituitary–adrenal system.

Effects of induction and hypnosis on the pituitary–adrenal system. A longitudinal protocol was developed to discriminate the effects of immobility from induction (IND) on pituitary–adrenal hormones, with their temporal pattern. The model (Table 1(b))—a single block of four trials during one day—included:

- Three immobility groups sacrificed at 0, 15, and 30 minutes after the last episode.
- Three induction groups: two groups sacrificed at 0 and 15 minutes, matched with immobility groups, with a period of time (empty time (ET)) after induction corresponding to the duration of hypnosis. Thus, the period between induction and sacrifice was the same in both the induction and the immobility groups.
- An additional group (00), sacrificed just after induction, represented the direct, immediate effect of induction per se, and the starting point of all other groups.

Corticosterone (C), adrenocorticotropic hormone (ACTH) and beta-endorphin were measured in plasma before the treatment (basal levels 24 hours before) and after the treatment at different times. Repeated measures were therefore available for each individual (before/after) (Farabollini et al., 1990).

Table 2 describes the hormonal modifications in both axes, following immobility and induction, starting from the first event in all groups (i.e. induction, group 00).

Table 2. Glucocorticoids and androgens modifications following immobility and induction

		Glucocorticoids	Androgens
Plasma	Induction 00	C ↑↑; ACTH ↑	
	IND + HYP + 0	C = ; ACTH ↑	
	IND + ET + 0	C ↑↑; ACTH =	
	IND + HYP + 15	C = ; ACTH ↑	T =
	IND + ET + 15	C ↑↑; ACTH =	T ↓↓
	IND + HYP + 30	C ↑; ACTH ↑	
Adrenal metabolites	Induction 00	C ↑↑; DOC =	T =; A =; 17a-OH-P=
	IND + HYP + 0	C =; DOC =	T =; A ↑; 17a-OH-P=
	IND + ET + 0	C ↑↑; DOC =	T ↓; A ↑; 17a-OH-P ↓
	IND + HYP + 15	C =; DOC =	T =; A =; 17a-OH-P=
	IND + ET + 15	C =; DOC ↑	T =; A =; 17a-OH-P ↓
	IND + HYP + 30	C ↑; DOC =	T =; A =; 17a-OH-P=

Note: ↑↑ individual (before/after) significant differences; ↑ slight significant differences vs. control; = not modified. The methodological characteristics of this study were: (a) a longitudinal study with subsequent time points; (b) repeated measures available for each individual (before/after); (c) groups 0 and 15 for both (IND + ET) and (IND + HYP) directly comparable, the period between induction and sacrifice being the same; (d) a starting point, i.e. the first event in all groups, represented by the group (00).

In plasma, C was mainly affected by induction; after an early increase, just after induction (group 00), a long-lasting effect and potentiation was found in groups (0) and (15). There was no recovery during the ET following induction.

When induction was followed by hypnosis, no significant difference was found in the corresponding (0) and (15) groups; this was indicative of a recovery during hypnosis, with respect to the effect of induction.

As for ACTH, after a slight increase just after induction (00), no difference was found in the (0) and (15) groups.

When induction was followed by hypnosis, significant increments, with a rapid onset (0) and long-lasting effects (groups 15, 30) were found. In parallel, there was an overall slight increase of plasma beta-endorphin in the immobility groups, significant only in group 15. ACTH and beta-endorphin are released from the common precursor, proopiomelanocortin, in the anterior pituitary; these modifications are congruent with the activation of an opioid mechanism in the immobility response, as found in pain studies (Carli, 1982).

On the whole, different effects of immobility and induction were found, with a dissociation on C and ACTH.

The marked increase of C after induction, with early onset and long-lasting effects, indicates the activation of adrenals after induction (in the presence of a slight increment of ACTH).

When induction was followed by immobility, there was a recovery in C levels, in the presence of a consistent, rapid, and long-lasting increment of ACTH.

On the basis of these findings, we considered the possibility that responsiveness of adrenals could be differently affected by induction and hypnosis.

Adrenal metabolism was therefore studied *in vitro*, using progesterone (P) as substrate (Lodi & Lupo, 1990) in the longitudinal model (Table 1(b)). In the adrenal gland, P is a common precursor of the corticosteroids biosynthesis and of adrenal androgens: the metabolites of the biosynthesis path of corticosteroids measured were C and 11-deoxycorticosterone (DOC).

In the induction groups, C biosynthesis increased in the first steps (00, 0), with a slight increase of the precursor DOC only at time 15. In the immobility groups, the lack of effects on the metabolites was indicative of a recovery towards normal values due to hypnosis.

The effects of induction and hypnosis on adrenal metabolism were in the same direction of plasma effects, proving the involvement of the peripheral component of the stress axis in these events.

HYPNOSIS AND PITUITARY–GONADAL SYSTEM

The effects of the two components of the treatment—immobility and induction—on testosterone (T) plasma levels were studied following a single episode, with samples taken 15 minutes after the end of the episode (Table 1(c)). Animals of the induction group were matched with the immobility group, with a period of time (ET) corresponding to duration of hypnosis, so that the delay from induction to decapitation was the same in the two groups (Farabollini et al., 1978).

The effect of induction was a decrease in T levels after 15 minutes, an expected effect in the frame of the stress-response described in the literature. No effect was found after immobility (see Table 2). Hypnosis, as in the case of corticosterone and its adrenal metabolites therefore induced a recovery to control levels, antagonizing the effect of induction.

T levels in plasma depend upon peripheral endocrine activity of gonads and adrenals, but also upon central control on the other end of the axis (i.e. anterior hypothalamus). In the hypothalamus, neural circuits controlling sexual behaviour are activated by T; however, just in those years the aromatization hypothesis was formulated indicating that in mammals the conversion of T to estradiol (E) through aromatization is a necessary step for the expression of male sexual behaviour (McEwen & Krey, 1984).

Adrenal metabolism was studied *in vitro*, using progesterone as substrate, in the longitudinal model (Table 1(b)), measuring the main metabolites of the biosynthesis path of adrenal androgens at different times after the last episode: 17-α-hydroxyprogesterone (17-alfa-OH-P), androstenedione (A) and T (Lodi & Lupo, 1990).

In the induction group (see Table 2), biosynthesis of T was reduced at time 0 with recovery after 15 minutes; the same pattern was found for 17-alfa-OH-P (the first metabolite of P, precursor of A and T), reduced at time 0 and 15 minutes later.

The effect of induction on androgen metabolites was in the same direction as plasma effects, with a transitory reduction followed by a rapid recovery after 15 minutes, and in an opposite direction with respect to that on corticosteroids.

The lack of effects of hypnosis on T and 17-alfa-OH-P was indicative of a recovery towards normal values, proving also in this case a mechanism of compensation of hypnosis with respect to induction.

Androstenedione was slightly increased both after induction and immobility at time (0), with a recovery after 15 minutes; the pattern was the same of plasma levels—increased at time (0) with a recovery at 15 minutes (not shown in Table 2).

A dissociation between induction and immobility, with hypnosis re-establishing the pre-induction balance, is therefore present in the case of adrenal androgenic metabolites (i.e. on a peripheral target organ).

Brain metabolism. The brain can be regarded as a steroid hormones target tissue for the activation of behaviour, in particular sexual behaviour. We studied the metabolism of T *in vitro* in the anterior hypothalamus using radioactive T as a substrate (Farabollini et al., 1978). In the model (Table 1(c)), two groups of immobility and induction only were matched so that the delay from induction to decapitation was the same in the two groups—animals were sacrificed 15 minutes following a single episode.

The main effect was a pronounced overall decrease of T metabolites, including dihydrotestosterone (DHT) and A, in the immobility group as well as in the induction group, but with a slighter effect. In fact, immobility potentiated the effect of induction, at least in the case of A. E formed *in vitro* from T (aromatization) was also strongly decreased in both induction and immobility groups.

The overall depression of T metabolism in the anterior hypothalamus found both following induction and hypnosis did not discriminate between the two components. In this respect, the metabolism at a central level differs from the metabolism in the peripheral tissue, as in adrenals. Hypnosis, unlike its effects on glucocorticoids, has no buffer action with respect to T central metabolism, if anything contributing to potentiate the effect of induction.

In order to evaluate if the reduced conversion of T to E and neutral metabolites in the hypothalamus was mediated by alterations in androgen binding, the T binding capacity was analysed in the amygdala (Lupo et al., 1994). The mechanism of androgens action for the control of sexual behaviour (but also of other sexually differentiated behaviours, such as agonistic behaviour), implies the binding to a specific protein in various brain areas (e.g. hypothalamus, preoptic area, and amygdala).

The model applied in this case was the longitudinal protocol (Table 1(b)), with three groups of immobility (0, 15, 30) and three groups of induction (00, 0, 15), matched as previously described.

A specific effect of immobility on T binding capacity, consisting in a decrease at 15 and 30 minutes after the end of the episode, with no effect of induction was found.

On the whole, hypnosis affects the T metabolism in the brain, with a general inhibition of the metabolic enzymatic systems and mechanisms in the same direction of induction. The reduced activity of aromatizing enzymes is of particular interest since the action of T on behaviour is mediated through its transformation into estradiol (McEwen & Krey, 1984).

The depression of T metabolism in the anterior hypothalamus following hypnosis could reflect the depression of sexual behaviour described in stressful situations. Sexual behaviour is in fact under the control of the anterior hypothalamus, with DHT and E acting synergistically to stimulate it in male rabbits (Beyer et al., 1975).

HABITUATION AND HYPNOSIS

The phenomenon of habituation (i.e. reduction of immobility duration following repeated episodes) is one of the constant characteristics of animal hypnosis; it is present in all species sensitive to the treatment and shows a similar pattern in different species (Lefebvre & Sabourin, 1977). Habituation is therefore a strong, reliable model to study the relative effects of induction and hypnosis, and to analyse the variable 'susceptibility'.

The protocol developed to obtain habituation (Table 1(d)) was a long-term treatment, as follows: repeated episodes of hypnosis in consecutive days (five trials per day) until habituation was reached. The criterion of habituation was when duration of immobility remained below 45 seconds in all the trials of two consecutive days; this was associated with the persistence of reduction in the next six days of treatment with induction.

Plasma corticosterone and ACTH were measured along the treatment at various intervals of the habituation protocol: weekly (on days 1, 8, 14), at habituation, and on the final day of treatment (Farabollini et al., 1981). In order to follow the temporal development of habituation, basal levels were measured (i.e. in blood samples taken just before the first daily trial).

All animals reached the criterion of habituation in different periods of time (mostly between days 8 and 14). Immobility duration was significantly reduced starting from day 6.

Our findings proved the process of habituation as being associated with the activation of the adrenocortical system. Plasma levels of C increased gradually during the treatment, with a peak at habituation and no significant recovery afterwards, when higher levels were maintained during the period of induction only.

A positive correlation between C concentrations and duration of habituation was found: the longer the time needed to reach habituation, the higher the levels of C. This was interpreted as a specific effect of the repeated exposure to the treatment (IND + HYP).

ACTH showed a tendency to increase at habituation, with a significant recovery afterwards. The increase of C plasma levels seemed to be specific to the development of habituation. During the treatment, the duration of hypnosis gradually diminished until it was almost abolished. As a consequence, in the block (IND + HYP) there was a gradual prevalence of IND, which was the only treatment left after habituation was reached. In this situation, effects of induction could be less and less compensated by hypnosis; this could explain the gradual increase of plasma C during the habituation process and its subsequent maintenance by the manipulative procedure of induction. In this process, ACTH likely works as a starter, activating adrenal cortex with a rapid recovery.

Habituation proved to be reversible following appropriate environmental modifications. In habituated animals, pain induced by formalin injection in the paw re-established susceptibility to hypnosis; this was coupled with typical EEG synchronization, if duration was long enough. The same effect was obtained through morphine injection (Carli et al., 1981). Pain (and morphine) effect on hypnosis duration in habituated animals were antagonized by the opioid antagonist naloxone. Incidentally, these results were further evidence of the activation of an endogenous analgesic morphine-like mechanism during hypnosis, as proved in another set of experiments.

When, after repeated treatment, animals reached habituation, they became unsusceptible (induced unsusceptibility). Since habituated subjects had high levels of C, persistent in the days following habituation, lack of susceptibility in these animals seemed to be a different

phenomenon from what is observed in animals naturally unsusceptible, unsusceptibility being associated to low levels of C.

In conclusion, the adrenocortical system is involved in susceptibility to immobility by restriction and inversion, in its various aspects, as spontaneous susceptibility and unsusceptibility induced through habituation. Re-establishment of hypnosis in habituated animals by alterations of the environment indicates that susceptibility may be manipulated with various procedures, in both directions.

Circulating sex hormones (T and E), were not affected by the process of habituation. However, when T metabolism and the conversion of T to E (aromatization) were measured in the anterior hypothalamus *in vitro* (incubation of the tissue with radioactive T as substrate), habituated animals were different from controls, with an overall increase of T metabolites in habituated animals, significant for DHT and E (Farabollini et al., 1981).

This long-term effect associated to habituation is different from the acute, short-term decrease of T metabolites in the hypothalamus 15 minutes after a single episode of immobility (Farabollini et al., 1978). On the whole, neuroendocrine modifications in habituated animals points to a re-arrangement of the stress-response system to a new 'steady state', with high levels of circulating corticosterone and increased sexual hormones metabolism in the anterior hypothalamus. This steady condition of different reactivity can be reversed, however, with appropriate stimuli, such as pain (Carli et al., 1981).

GENERAL CONCLUSIONS

This is a report of experiments on the neuroendocrine aspects of animal hypnosis in rabbits, proving the involvement of the pituitary–adrenocortical system and pituitary–gonadal system and allowing us to draw some conclusions on the function of animal hypnosis.

Peripheral modifications of corticosterone and testosterone plasma levels following induction were typical of the stress-response, with a marked and long-lasting (at least until 15 minutes after the end of the episode) increase in C, and a parallel reduction of T. Similar findings were obtained with physical restraint, one of the most common stressors, confirming the nature of induction as an aversive stressful event (Porro & Carli, 1988).

When induction was followed by immobility, there was a recovery of both hormones to control levels within 15 minutes; on the contrary, there was no recovery during the empty time (matched with hypnosis duration) following the induction.

The hypothesis is that hypnosis functions as a sort of buffer system, re-establishing the endocrine balance with respect to the effects of the stressful event, represented by the manipulative procedure of induction. This interpretation is supported by the electrophysiological pattern of response during hypnosis, with EEG synchronization.

The compensatory effect of hypnosis is likely to work at the peripheral level; a dissociation between induction and immobility, with hypnosis re-establishing the pre-induction balance, was present also in the adrenals, where the metabolites of both the biosynthesis path of corticosteroids and of adrenal androgens were modified accordingly to plasma alterations.

The finding of a depression of T metabolites in the anterior hypothalamus following hypnosis, in the same direction of induction, suggested that the recovery function of hypnosis is limited to the stress-response system.

The hypothalamus, perhaps the most interesting part of the brain to behavioural endocrinologists, is the site where hormones activate mating behaviour in adult male vertebrates. In this area, the action of testosterone on sexual behaviour was found to be mediated through its transformation into estradiol by the enzyme aromatase (McEwen & Krey, 1984). It was also proved that the neural circuits controlling behaviour were different from those involved in the hypothalamus–pituitary–gonadal axis, through gonadotropin-releasing hormone, and the cascade of luteinizing hormone and follicle-stimulating hormone, stimulating peripheral secretion of T (Heimer & Larsson, 1967). Therefore, T plasma levels do not necessarily reflect T brain metabolism and aromatization.

The depression of T metabolism could reflect a depression of sexual behaviour described in stressful situations. This raises the question of the possible relation between hypnosis and mating. In other experiments carried out in our lab on amphibia, susceptibility to hypnosis by inversion was related to sex and the condition of mating, with parallel modifications of sex hormones (Lupo et al., 1987).

Susceptibility to hypnosis could be modified, and almost eliminated, through a prolonged repetition of episodes leading to habituation. In habituated animals there was a rearrangement of the stress-response system to a new 'steady state' with higher circulating corticosterone; this condition can be reversed by appropriate stimuli, such as pain (Carli et al., 1981).

Looking back, after so many years, to these experiments on animal hypnosis, I realize how interesting the subject was and how many questions remained unanswered. Our group then scattered towards different research fields; however, most of us are still linked by personal friendship and affection. As for Giancarlo Carli, he has played an important role in my scientific career, and it was most fortunate that we could rely one on the other all through our lives.

REFERENCES

Beyer C, de la Torre L, Larsson K, Perez Palacios G (1975). Synergistic actions of estrogen and androgen on the sexual behavior of the castrated male rabbit. *Hormones and Behavior* 6: 301–306.

Carli G (1982). Animal hypnosis: an attempt to reach a definition. *Archives Italiennes de Biologie* 120: 138–159.

Carli G, Farabollini F, Fontani G (1981). Effects of pain, morphine and naloxone on the duration of animal hypnosis. *Behavioural Brain Research* 2: 373–385.

Carli G, Farabollini F, Lupo C (1979). Plasma corticosterone and its relation to susceptibility to animal hypnosis in rabbits. *Neuroscience Letters* 11: 271–274.

Farabollini F, Facchinetti F, Lupo C, Carli G (1990). Time-course of opioid and pituitary-adrenal hormone modifications during the immobility reaction in rabbits. *Physiology and Behavior* 47: 337–341.

Farabollini F, Lupo C, Carli G (1978). Changes in plasma testosterone and in its hypothalamic metabolism following immobility responses in rabbits. *Physiology and Behavior* 20: 613–618.

Farabollini F, Lupo C, Carli G (1981). Neuroendocrine changes following habituation of animal hypnosis in male rabbits. *Behavioural Brain Research* 2: 363–372.

Heimer L, Larsson K (1967). Impairment of mating behavior in male rats following lesions in the preoptic-anterior hypothalamic continuum. *Brain Research* 3: 248–263.

Lefebvre L, Sabourin M (1977). Response differences in animal hypnosis: a hypothesis. *The Psychological Record* 1: 77–87.
Lodi L, Lupo C (1990). Modificazioni adrenocorticali in seguito alla reazione di immobilità nel coniglio. *Accademia Fisiocritici Siena* XV: 127–134.
Lupo C, Lodi L, Farabollini F (1994). Testosterone binding in the rabbit amygdale after the immobility reaction. *Medical Science Research* 22: 707–708.
Lupo C, Lodi L, Paluffi G, Viti A (1987). Tonic immobility and testosterone receptors in the brain of mating and non-mating toads. *Monitore Zoologico Italiano* 21: 191–192.
McEwen BS, Krey LC (1984). Properties of estrogen sensitive neurons: aromatization, progestin receptors induction and neuroendocrine effects. In Celotti F, Naftolin F, Martini L (eds) *Metabolism of Hormonal Steroids in the Neuroendocrine Structures*. New York: Raven Press, pp. 75–83.
Porro CA, Carli G (1988). Immobilization and restraint effects on pain reactions in animals. *Pain* 32: 289–307.

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