

Photoperiodic Signalling Through the Melatonin Receptor Turns Full Circle

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Photoperiod exerts profound influence on the physiology of mammals through the action of melatonin on the neuroendocrine system. Over the last 20 years, studies have moved away from a melatonin receptor-focused approach to understanding how photoperiod regulates neuroendocrine activity through studies of downstream effects on gene expression. This paper reviews the recent progress made in our understanding of the effects of photoperiod on gene expression in the hypothalamus, and considers how this new information can be reconciled with the species-specific location of melatonin receptors.

Key words: melatonin, thyroid, retinoid, parastuberalis, kisspeptin, prolactin.

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Seasonal cycles of environmental change are a feature of habitats from the tropics to the poles, and life adapts to these through corresponding changes in behaviour or physiology. The predictable annual cycle of changing day length (photoperiod) is a major synchronising cue for seasonal adaptation, and allows organisms to express adaptive responses in anticipation of changes in environmental favourability. In mammals, photoperiodic information is relayed through the secretion of the hormone melatonin by the pineal gland, which then acts on the neuroendocrine system to produce adaptive changes in endocrinology, anatomy and physiology, affecting reproduction, moulting, energy balance and behaviour. In the 20 years since the founding of the *Journal of Neuroendocrinology* there has been great progress in our understanding of the role of melatonin in mammalian photoperiodic timing; this and future prospects are the focus of this article.

The term 'synchronising' implies that the external photoperiod interacts with endogenous long-term timing processes to achieve appropriately timed seasonal responses. In mammals, and in birds, which are less dependent upon melatonin for photoperiodic responses (1), these long-term timing processes become evident when animals are held on artificially constant photoperiods for extended periods. In some cases (e.g. sheep, ground squirrels and stonechats), photoperiodic clamping reveals expression of recurrent cycles of seasonal physiology and behaviour, which may persist for a decade or longer (2, 3); so-called 'circannual rhythms'. In other cases, endogenous long-term timing is limited to an endogenously driven transition to a new seasonal state, after which further endogenous changes do not occur [e.g. in hamsters reversion to a summer breeding phenotype, following short photoperiod

(SP)-induced gonadal regression (4)]. This latter phenomenon has been described as 'photorefractoriness', but this term can be misleading as photorefractory mammals remain fully light- and melatonin-responsive at the level of the pars tuberalis (PT) and in the suprachiasmatic nucleus (5, 6). Such endogenous long-term timing is probably of adaptive value for animals during winter hibernation or around the solstices, when photoperiodic change is hard to detect. A comprehensive model for melatonin action in seasonal mammals must account for these long-term timing processes, the mechanisms whereby they become synchronised to the pineal melatonin signal, and the breakaway of downstream physiology occurring during exposure to constant photoperiod.

The pineal gland secretes melatonin at night as a continuous signal whose duration is proportional to the night length (7). Pinealectomy experiments, combined with timed replacement of melatonin by infusion pumps, demonstrated that photoperiod-dependent changes in the melatonin signal duration convey the seasonal message in mammals (8). Despite this insight, largely established by the late 1980s, understanding of melatonin action at the cellular level was hindered by lack of knowledge of the nature and anatomical location of melatonin receptors. (9).

In 1987 the first visualisation of melatonin receptors in the brain was achieved using the radioligand 2-¹²⁵I-iodomelatonin (10), and this breakthrough sparked an intense period of study to find the sites of action in the brain, which might explain the photoperiodic effects of melatonin. Several striking conclusions emerged from these studies. Firstly, melatonin receptor expression in the mammalian brain was remarkably species-specific (9). This finding went against expectation, as it was anticipated that melatonin receptors

at common sites in the hypothalamus would explain many of the photoperiodic effects of melatonin; the lack of consistency across species was a puzzle. Secondly, melatonin receptors were found in the PT of the pituitary (11, 12) and it was soon recognised that this site was strongly conserved throughout seasonal mammals (9). Thirdly, in contrast to the rather limited distribution of melatonin receptor expression within the mammalian brain, melatonin receptors had widespread expression in the brains of birds and other lower vertebrates (13).

Two-site hypothesis for seasonal effects of melatonin

Some elegant hypothalamo-pituitary disconnection experiments in Soay rams by Lincoln *et al.* (14, 15) have implicated the PT in the seasonal control of prolactin secretion, by a mechanism independent of the hypothalamus. In this tissue, melatonin exerts a range of acute and duration-dependent effects of melatonin on cAMP-based signalling and on clock gene expression, which probably allow for durational interpretation of the melatonin signal (16–19).

In contrast to prolactin, lesioning and microimplantation experiments in hamsters and in sheep suggest that melatonin controls seasonal changes in reproduction and energy balance through basal hypothalamic sites (dorso/ventromedial hypothalamic nuclei and the premammillary/arcuate region) (20–25). Here, melatonin receptor-expressing cells have not been characterised, and consequently the recent focus has been on examining hypothalamic gene expression profiles as indicators of photoperiodic response and sites of action.

The role of intrahypothalamic thyroid hormone metabolism in the photoperiodic response

The possibility that thyroid hormone (TH) plays a key role in the expression of seasonal cycles of physiology dates back over six decades to studies showing that the reproductive rhythm of starlings is abolished by thyroidectomy (26). Subsequent work in a range of avian and mammalian species supported this idea and established a link between thyroid function and the termination of breeding (27). Work in sheep, using local implants of thyroxine (T4) in thyroidectomised ewes, showed that this spring TH requirement was localised to sites in the basal hypothalamus (28). Nevertheless, in the absence of consistent photoperiod-dependent changes in TH titres measured in the circulation, it has remained unclear how TH signalling could assume anything beyond a permissive role in seasonal timing mechanisms.

This view was overturned by studies in the Japanese quail by Yoshimura *et al.*, who demonstrated that photoperiod profoundly influences local hypothalamic levels of triiodothyronine (T3). This is attributable to photoperiodic effects on the relative expression of type II and type III deiodinases (DIO2 and DIO3, respectively) in the basal tuberal hypothalamus, DIO2 increasing and DIO3 decreasing rapidly upon transfer of quail to long photoperiod (LP) (29, 30). These enzymes exert opposing effects on TH metabolism, DIO2 acting to promote conversion of T4 to T3, which is a more potent activator of TH receptors, while DIO3 converts T4 to the biologically inactive form reverse T3 (Fig. 1). LP induction of DIO2 expression

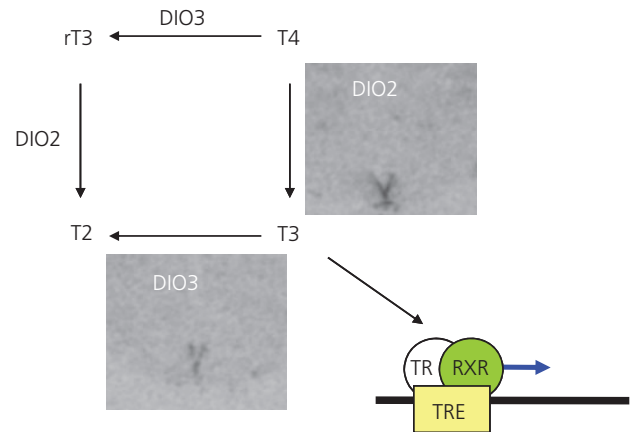


Fig. 1. The enzymatic metabolism of thyroid hormones. Triiodothyronine (T3) is the biologically active form of thyroid hormone. This activates the nuclear thyroid hormone receptor (TR) which dimerises to the retinoid X receptor (RXR) to regulate gene expression. T3 is formed by conversion from thyroxine (T4) by the type II deiodinase enzyme (DIO2). T3 can be catabolised to T2 by the enzyme type III deiodinase (DIO3). Both DIO2 and DIO3 are expressed in the ependymal cells of the third ventricle in the hypothalamus. The gene expression of one, or both, enzymes is regulated by photoperiod, depending upon species. DIO3 also regulates the catabolism of T4 to reverse (r) T3, and DIO2 regulates its subsequent metabolism to T2. Autoradiographs show the expression of DIO2 and DIO3 in the ependymal layer of the third ventricle of the hypothalamus in Siberian hamsters. TRE, thyroid hormone response element.

was associated with an approximately eightfold increase in T3 in the quail hypothalamus, without corresponding changes in circulating T3 titres (29). Finally, central injection of T3 and inhibition of DIO2 activity positively and negatively regulate luteinising hormone (LH) secretion, respectively, indicating that the intrahypothalamic T3 level is a key determinant of reproductive activation in the quail.

Similar photoperiodic effects on deiodinase gene expression have now been reported in photoperiodic mammals, and shown to be melatonin-dependent, suggesting an evolutionarily conserved mechanism across vertebrates (31–35). Further, in Siberian hamsters, chronic-release T3 microimplants placed into the hypothalamus prevent animals from undergoing SP-induced testicular regression and loss in body weight as compared with sham-operated controls (35). This striking observation is consistent with the hypothesis that a decline in hypothalamic T3 is required to permit the SP response, possibly as a consequence of the increase in DIO3 gene expression and enzyme activity observed in this species following transfer from LP to SP (35, 36).

In mammals, variable long-term timing or photoperiodic history effects on TH-related signalling components, including the deiodinases, have also been reported. In the Siberian hamster, short day-induced expression of DIO3 spontaneously attenuates with prolonged exposure to short days, and this precedes gonadal reactivation (35, 36). For DIO2 in this species, the picture is complex: in young animals raised on SP, subsequent LP exposure promotes expression of DIO2, whereas in adults acclimated to LP, a switch to SP fails to suppress DIO2 expression. This contrasts with the situation in adult Syrian hamsters, in which SP exposure suppresses

DIO2 expression, and this is maintained during prolonged SP exposure, even when animals spontaneously reactivate the gonadal axis following prolonged SP exposure.

Further complexity is suggested by a microarray analysis in Siberian hamsters, in which prolonged SP exposure has been associated with a decline in expression of genes for the TH-binding proteins, transthyretin, thyroid-binding globulin and albumin (37). The presence of the organic ion transporter genes *cOatp1c1* (chicken organic anion transporter protein) and *cOatp1b1*, which can act as TH transporters, has been observed in the ventral lateral walls of the basal tuberal hypothalamus of quail, but here no photoperiodic difference in the expression pattern was detectable (38). Collectively, these observations suggest that local regulation of TH signalling within the hypothalamus is more complex than acute stimulatory or inhibitory effects in DIO2/DIO3, and is probably involved both in primary photoperiodic responses and in delayed long-term timing effects.

The location and nature of deiodinase-expressing cells

Hypothalamic deiodinase gene expression is concentrated to the ependymal cell layer surrounding the bottom of the third ventricle (29, 31–35), and descending into the median eminence; this area comprises a specialised population of cells known as tanycytes (39). These cells form projections into the external zone of the median eminence, and have been variously implicated in the bidirectional uptake and transport of molecules across the blood–brain barrier, and in controlling the access of hypothalamic neurosecretory cell endings to capillaries in the primary plexus of the portal system (39). Additionally, the ependymal layer is a site of cell proliferation in the brain of adult rats (40). Effects on TH availability mediated by deiodinase gene expression might influence any of these processes – or the function of TH-sensitive cells in neighbouring hypothalamic regions.

The dorsal medial posterior region of the arcuate nucleus (dmpARC) as a photoperiodically sensitive hypothalamic nucleus

Microarray and candidate gene profiling suggest that a subregion of the dmpARC is one structure through which downstream effects of altered TH levels may be channelled (41, 42). The dmpARC is a distinct cell cluster evident in the ARC of the Siberian and Syrian hamsters, but is less well defined in either laboratory rats or mice (43) (Fig. 2). Thus whether the dmpARC is a specialisation, specific to some or all photoperiodic species, remains to be established. Given the close proximity of the ependymal layer and the dmpARC it is likely that there is a functional relationship. Indeed, immunostaining against the protein vimentin in Djungarian hamsters has revealed the extensive projections that radiate from the tanycytes into the adjacent medial basal hypothalamus, and it has also been shown that these projections are photoperiod-dependent (44).

The dmpARC expresses TH receptors (TRs), and shows photoperiod- (and melatonin-) dependent expression of the retinoic acid receptor (RAR), cellular retinoic acid-binding protein 2 (CRABP2)

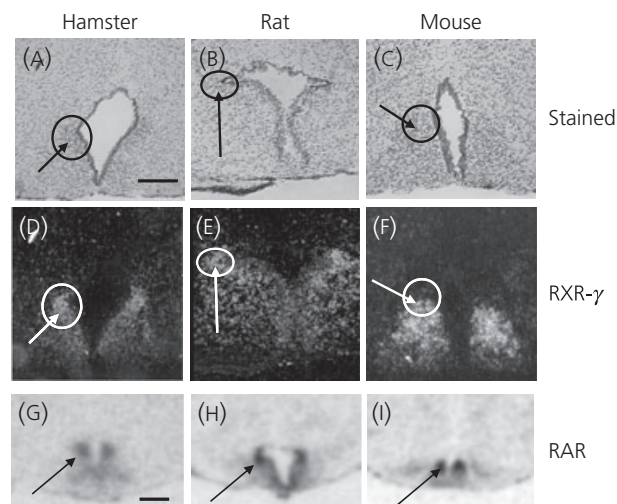


Fig. 2. The dorsomedial posterior arcuate nucleus (dmpARC) of the hypothalamus, shown in toluidine blue-stained sections from the Siberian hamster (A), Wistar rat (B) and Aston mouse (C). Note that the cell cluster defining the dmpARC is more evident in the hamster hypothalamus. (D–F) Retinoid X receptor (RXR)- γ gene expression detected by *in situ* hybridisation in the hypothalamus of Siberian hamster, Wistar rat and Aston mouse, respectively. Slides show silver grains overlying cells in the dmpARC of the hypothalamus. (G–I) Gene expression of the retinoic acid receptor (RAR) in the dmpARC of the Siberian hamster, Wistar rat and Aston mouse detected by *in situ* hybridisation. Note that gene expression for RAR in dmpARC is more discrete than for RXR in mouse, which is expressed more widely in the main ARC. Arrows indicate dmpARC. Scale: bar for (A–F), 200 μ m; bar for (G–I), 500 μ m.

and the retinoid X receptor (RXR) (41). As RXR can form heterodimers with both RAR and TR (45), there is the potential for interaction between the thyroid and retinoid pathways. Interestingly, cellular retinol-binding protein 1 (CRBP1), which functions as a cellular binding protein and transporter of retinol, is expressed in the ependymal layer (41). This could mediate delivery of retinoic acid to the dmpARC, where CRABP2 may facilitate the enzymatic conversion of retinol to retinoic acid and its onward delivery to RARs. Hence ependymal cells may govern a photoperiod-dependent supply of ligands for both TRs and RARs in adjacent hypothalamic sites. So far, however, efforts to demonstrate a role of retinoid signalling in seasonal responses, using RAR agonists or antagonists, have been unsuccessful (F. Ebling, pers. comm.).

At present neither the downstream targets of the retinoid nor thyroid receptors in the dmpARC neurones have been identified. It is known that the histamine H3 receptor as well as the non-acronymic secretory peptide VGF and proteolytic cleavage enzyme proconvertase enzyme 2 (PC2) are expressed and photoperiodically regulated in the dmpARC (42, 46). Whether there is a functional relationship between the retinoid and thyroid receptors and the expression of these genes is an interesting area for future study.

Kisspeptin and RF-amide related peptide

Recent studies in Syrian and Siberian hamsters have implicated members of the RF-amide family as downstream players in photoperiod signalling through melatonin (47, 48). Kisspeptin, a 52-amino

acid peptide product derived from a precursor protein product of the KiSS (kisspeptin)-1 gene, is known to be involved in the regulation of the gonadotrophic hormone-releasing hormone (GnRH) through GPR54 receptors, which are expressed on GnRH neurones (49, 50). The KiSS-1 gene is expressed in two main populations of cells in the hypothalamus: in the anteroventral periventricular nucleus (AVPV) and in the ARC (47, 51, 52). Studies in male Syrian hamsters have shown that, relative to reproductively active animals maintained in LP, expression of the KiSS-1 RNA/protein is suppressed in the ARC in hamsters following transfer to SP, leading to reproductive quiescence (47). Continued SP exposure, leading to spontaneous gonadal reactivation, also leads to restored ARC KiSS-1 expression (47). These effects are melatonin-dependent and not secondary to altered steroid feedback as a function of reproductive state, and intracerebroventricular kisspeptin-10 infusion to SP hamsters restored reproductive activation (47).

A slightly different picture has been presented in Siberian hamsters, with reciprocal effects of photoperiod in the AVPV compared with the ARC; SP suppressing peptide expression in the former and increasing it in the latter (53). Additionally, intraperitoneal injection of kisspeptin-10 increased LH secretion only in LP-housed animals (53). Further studies are needed to assess the extent to which these apparent differences reflect true species differences or differing experimental approaches – peripheral peptide administration and reliance on immunocytochemistry alone (53) versus central peptide delivery and combined immunocytochemistry and *in situ* hybridisation (47).

In sheep, kisspeptin also varies with season (54) and photoperiod (19), with reduced ARC expression occurring in animals transferred from SP to LP (19). Hence there appears to be a conserved involvement of altered kisspeptin signalling in seasonal reproductive responses in mammals. While this might represent a pathway that is independent of either the thyroid or retinoid responses, it seems likely that it lies downstream of, and is dependent upon, these signals.

Kisspeptin is a member of the RF-amide family of peptides, which in mammals are derived from five different genes [designated F-amide related peptides (farp)1–5], for which five cognate G-protein coupled receptors have been identified [designated RF amide receptor, (rfr)-1–5] (55). A recent study has examined whether another member of this family, the gene encoding RF-amide related peptide (RFRP), is involved in photoperiodic signalling. This gene is interesting, as RFRP has been implicated as a gonadotrophin inhibitory hormone (GnIH) in birds and in mammals (56, 57). In both Syrian and Siberian hamsters, RFRP gene expression is localised to an area between the ventromedial hypothalamus (VMH) and dorsal medial hypothalamus (DMH), and it is photoperiodically regulated. In hamsters maintained in SP for 10 weeks, RFRP is less than 30% of the expression in LP, and this effect is pineal/melatonin-dependent (48).

The physiological function of RFRP is not clear. While RFRP3 has been shown to suppress plasma LH levels when injected intracerebroventricularly into both rats and Syrian hamsters (57, 58), consistent with orthologous function to GnIH in birds, the reduced expression of RFRP in SP-housed Syrian hamsters, in which the gonadotrophic axis is fully suppressed, seems at odds with such an

inhibitory role (48). Similarly, hypothalamic RFRP expression is higher under LP in sheep (D.G. Hazlerigg, unpublished observations), suggesting that a conserved link between RFRP and seasonal reproduction does not exist. One other possibility that needs further evaluation is that RFRP may play a role in prolactin regulation, as, following its initial isolation, it has been shown to inhibit dopaminergic control of prolactin in the rat (59).

Integrating hypothalamic responses with melatonin action: the two-site hypothesis revisited

The outstanding question regarding the photoperiodic control of hypothalamic gene expression is how these events are linked to melatonin receptor pathways. Lesioning and implant experiments have been interpreted to favour a two-site model of melatonin action, where basal hypothalamic targets are linked to reproduction, yet the PT governs seasonal plasma prolactin levels (Fig. 3A)

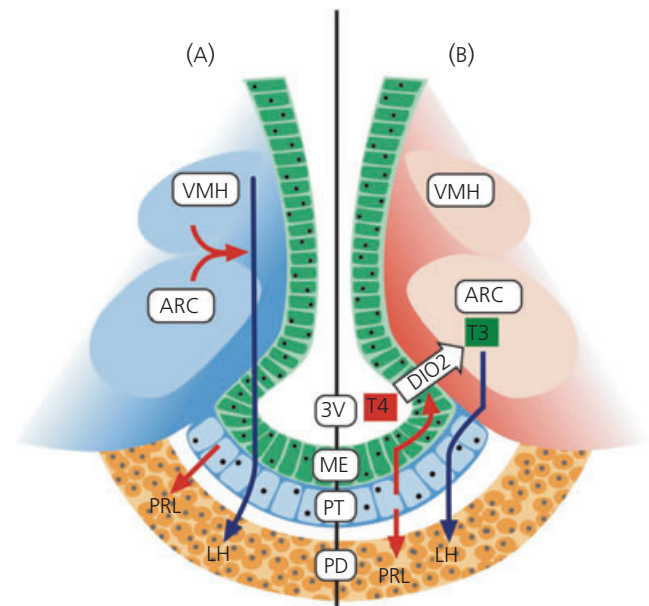


Fig. 3. The dual sites of melatonin action hypothesis revisited. Shown is a schematic of the hypothalamic system in coronal view, with two alternative views (A and B) of the photoperiodic effects of melatonin presented to either side of the midline. View (A): the blue-shaded regions indicate presumptive sites of melatonin action, with red arrows indicating directly melatonin-regulated signals and blue arrows indirect consequences of these signals. This 'classical' dual site of action view is based on lesioning and melatonin-microimplantation experiments. Here melatonin is presumed to act via the pars tuberalis (PT) to govern prolactin secretion by the pars distalis (PD), and via the ventromedial or basal hypothalamus to control reproductive hormone secretion and energy homeostasis. View (B): a revised view, placing thyroid hormone deiodinase gene regulation at the centre of hypothalamic responses to melatonin. It is hypothesised that control of type II deiodinase (DIO2) depends on a melatonin-dependent signal relayed by adjacent PT cells (blue region), while the same, or an additional, PT-dependent signal accounts for control of prolactin secretion, as in (A). VMH, ventromedial hypothalamus; ARC, arcuate nucleus; 3V, third ventricle; ME, median eminence; PRL, prolactin; LH, luteinising hormone; T3, triiodothyronine; T4, thyroxine; DIO, deiodinase.

(20–25). Nevertheless, such studies leave room for alternative interpretations. This is because we cannot exclude the possibility that lesions destroy downstream sites of the melatonin-responsive pathway, nor that melatonin diffusion away from sites of microimplant placement reaches biologically significant levels. These concerns are particularly pertinent for a region of such complex local anatomy as the hypophyseal neuroendocrine system, and must be kept in mind.

Corroboration of these functional anatomical tests through analysis of melatonin receptor distribution does nothing to alleviate these concerns: although hypothalamic binding of iodomelatonin is documented and much relied upon (9), this encompasses binding to molecules unrelated to melatonin signal transduction (60), and expression of melatonin receptor subtypes in the mediobasal hypothalamus which are undetectable by *in situ* hybridisation. Hence, for the recently documented photoperiodic control of deiodinase gene expression in periventricular cells, we must either accept the possibility of melatonin action through receptors at these sites, which so far remain undetectable, or consider the role of neighbouring sites, serving a relay function. Inevitably this leads to a reconsideration of the role played by the PT (Fig. 3b).

The pituitary disconnection studies that drew Lincoln and Clarke (14) to link the PT to seasonal prolactin secretion suggest that the PT may secrete a prolactin-releasing factor (PRF), which acts locally within the anterior pituitary. Although several studies have described the existence of PRF activity in PT cell extracts (5, 61, 62), the identity of this remains elusive. Furthermore, the putative existence of a PRF does not exclude the additional possibility that PT products also act upon neighbouring cells in the median eminence. This idea is not new (9, 63), but is given renewed impetus by the new data on deiodinase regulation. In recent pioneering work on quail, Yoshimura and colleagues have demonstrated early photoinduction of thyrotrophin- β (TSH- β) mRNA in the PT as well as thyroid-stimulating hormone (TSH)-dependent regulation of DIO2 gene expression in the cells of the ependymal layer (64). This provides strong evidence to support the view that the PT plays a critical role in linking photoperiod to downstream events involving the hypothalamus in the quail. While it remains to be shown whether this mechanism also holds true for mammals, it nonetheless seems likely, on the basis of conserved photoperiod-dependent changes in deiodinase expression in the ependymal layer of birds and mammals. These studies also leave open the tantalising possibility that TSH may be a common hormonal mediator of the photoperiodic responses through the hypothalamus as well as pituitary (i.e. prolactin). The nature of the communication between the PT and the cells of the ependymal layer as well as the pituitary in mammals is now a clear research priority.

And so in two decades the wheel turns full circle. The first article to appear in the *Journal of Neuroendocrinology* described the presence of melatonin-binding sites in the PT (12), and hypothesised that it was a key site for seasonal photoperiodic responses. Subsequent work eroded this position to a limited role specifically in prolactin regulation (14). Now, as the *Journal* enters its third decade, the hypothesis that the PT is the principal site through which melatonin

controls hypothalamic function, and hence reproduction and metabolism, must again be seriously considered.

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