Intrinsic processing in the mammalian superior colliculus
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The mammalian superior colliculus (SC) receives visual inputs from the retina and primary visual cortex in its superficial layers and sends descending motor commands from its deeper layers. It is now becoming clear that a connection exists between these layers, but the signal transmission through it is not robust. The induction of burst discharges in the deeper layer neurons by direct visual inputs from the superficial layers may lead to 'express' saccadic eye movements with extremely short reaction times in behaving animals.

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Abbreviations
AChe acetylcholine
AMPA α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP5 D-2-amino-5-phosphonovalerate
Bic bicuculline
CNQX 6-cyano-7-nitroquinoline-2,3-dione
dSC deeper layers of the superior colliculus
EPSP excitatory postsynaptic potential
IPSP inhibitory postsynaptic potential
NMDA N-methyl-D-aspartic acid
OT optic tract
SC superior colliculus
SGS stratum griseum superficiale
SGI stratum griseum intermediale
SO stratum opticum
SRT saccadic reaction time
sSC superficial layers of the SC

Introduction
The mammalian superior colliculus (SC) is a brainstem centre that controls orienting behaviours of various parts of the body, such as the eyes, head, trunk and pinnae [1,2]. The superficial layers of the SC (sSC), comprising the superficial gray layer of the SC (the stratum griseum superficiale [SGS]) and the optic layer of the SC (stratum opticum [SO]), receive visual inputs from the retina and the primary visual cortex and send output signals to the pretectal regions. In the sSC, the contralateral visual hemifield is represented in a topographically organised map [3].

By contrast, the deeper layers of the SC (dSC), comprising the intermediate and deep layers of the SC, receive inputs from nonvisual sensory modalities, frontal and parietal cortical regions and basal ganglia and send descending and/or ascending signals to the brainstem reticular formation, spinal cord and thalamus [4]. Whereas much interest has been directed toward the input–output organisation of the SC, very little is known about the neural connections and signal processing that are intrinsic to the SC.

In this review, I summarise current knowledge and recent research on the intrinsic signal processing in the local circuits of the SC. In particular, I discuss results that strengthen my hypothesis that the dynamic modulation of signal transmission occurs through a vertical interlaminar connection between the sSC and dSC [5] and discuss neuronal mechanisms for generating the saccade-related burst activity of the dSC neurons.

Activities of collicular neurons during express and regular saccades
As first described by Fischer et al. [6,7], the probability of the occurrence of saccades with an extremely short (80–120 ms) saccadic reaction time (SRT), called 'express saccades', increases markedly when a time interval lasting for a few hundred milliseconds separates the fixation offset and appearance of a novel target — an interval we call the 'gap period' [6,7]. Interestingly, SRTs have a bimodal distribution: SRTs of express saccades have an additional peak as compared with the longer SRTs (150–250 ms) of 'regular saccades', which are more frequently observed when no gap period is given to the subject ('no-gap' condition). The probability of the occurrence of express saccades depends on experimental parameters such as gap duration, practice, predictability and stimulus sequence [8,9]. Such bimodality has led us to examine the neural pathways involved in the sensory-motor transformation of 'express' and 'regular' saccades.

Figure 1a shows the discharge of an sSC neuron and a dSC neuron in monkey for 'regular saccades' (SRTs > 150 ms) in the no-gap condition, whereas Figure 1b shows the discharge of the same neurons for express saccades (in a gap task with a gap period equal to 170 ms). It can be seen that the discharge of the sSC neuron is virtually the same for regular and express saccades: in both cases, it shows phasic visual responses, with latencies of 40–50 ms. Thus, the execution of express saccades cannot be ascribed to differences in the discharge of sSC neurons.

By contrast, the dSC neuron does not show the same pattern of activation for the two types of saccades. For regular saccades, the dSC neuron shows a weak discharge with a latency of 40–50 ms (i.e., similar to that of the visual responses of sSC neurons). This weak visual response does not seem to be strong enough by itself to trigger the saccade burst generator circuits that lie downstream of the SC [10,11]. But after 50–80 ms, the dSC neuron shows a strong burst of spikes (a 'saccade-related burst'), which seems to trigger saccades [12].

For express saccades in the gap condition, a gradual increase in activity called ‘build-up activity’ is observed...
Neuronal activity of collicular neurons in cases of regular and express saccades (a) Activity of an sSC neuron and a dSC neuron during regular saccades in the ‘no-gap’ model. (b) Express saccades in the gap model (with a gap period of 170 ms). Spike density functions of exemplary neurons are shown. Eye, eye position; FP, presentation of fixation point; ST, presentation of saccade target. (c,d) Current hypothesis of the signal flow in regular saccades (c) and express saccades (d). Red circles and lines indicate high activation. Top panels (c1,d1) show the activation of individual regions at the time when the visual input reaches the superficial layers. Bottom panels (c2,d2) indicate the signal flow to induce saccades. In (c1), the activity level of the dSC neuron is low; therefore, the visual response of the sSC neuron can induce only a small and phasic visual response in the dSC neuron. Instead, the signal passing through the cortical structures induces bursts in the dSC neurons to induce ‘regular saccades’ later (c2). In (d1), the activity level of the dSC neuron is enhanced by preparatory input (a half-tone of dSC, corresponding to the build-up activity in [b]); therefore, the signal flows directly from the sSC to the dSC, inducing ‘express saccades’ (d2). Note the half-tone circles representing the lateral geniculate nucleus (LGN), pulvinar (PL) and frontal eye field (FEF), supplementary eye field (SEF) or lateral inferior parietal area (LIP), which indicate that the contribution of these structures to the execution of express saccades is not clarified. It has been reported that FEF neurons show enhanced activation during the gap model [73,74].

during the gap period (Figure 1b, arrow). The build-up activity is followed by a strong unimodal burst of spikes with a latency equal to that of the ‘visual responses’. In the gap condition, this unimodal burst seems to trigger saccades — an observation that is consistent with previous studies showing that the visual and motor bursts fuse in dSC neurons to trigger express saccades [12-14]. It has been reported that SRTs are correlated negatively with the
firing frequency of the build-up activity that immediately precedes the visual response to presentation of the visual target [14]. Thus, the data also suggest that when dSC neurons show higher build-up activity, their weak visual responses (Figure 1a) can be transformed into bursts strong enough to trigger saccades.

On the basis of these observations of the neuronal activities in the sSC and dSC, I propose the following model for the underlying neuronal mechanisms (Figure 1c). If the activity level of the dSC neurons is low at the time of visual response to the target presentation, then the visual input that is mediated directly through the sSC causes only a
weak visual response in the dSC neurons that does not surpass the threshold for inducing saccade-related bursts (Figure 1c, 1). In this case, the signal is processed through cortical structures and the saccade-related bursts in the dSC neurons are induced by the cortical inputs that reach the dSC later (Figure 1c, 2). However, if the activity level of the dSC neurons is high enough at the time of visual response (Figure 1d, 1), then the visual input from the sSC can surpass the threshold for inducing saccade-related burst and lead to the direct execution of express saccades (Figure 1d, 2).

This hypothesis is based on the two neuronal mechanisms described below, however, it also necessitates additional arguments: first, that there is an interlaminar connection between the superficial and deeper layers of the SC; and second, that there is an ‘all-or-none’ gating mechanism for inducing the burst activities in the dSC neurons.

The interlaminar connection and its functional implication

Previous arguments

Robinson [15] first demonstrated the vectors of saccades evoked by electrical stimulation and used these to describe a topographical map of saccade vectors in the deeper layers of the SC. In the same year, Schiller and Stryker [16] demonstrated that the motor map is in register with the map of visual space that is represented in the superficial layers. On the basis of these findings, it was proposed that the superficial and deeper layers of the SC are organised in a columnar fashion by interlaminar connections that link them [17]. In support of this, Maeda et al. [18] showed that short-latency excitatory postsynaptic potentials (EPSPs) can be induced in stratum griseum intermediale (SGI) neurons after electrical stimulation of the optic nerve in anaesthetised cats.

But the existence of such a connection and its functional implications have been debated at length in the field of oculomotor research. Edwards [19] presented evidence based on anatomical inspection against the existence of interlaminar connections. In addition, Mays and Sparks showed that during certain saccades, such as the second saccade in the double-step saccade model, the bursting discharges of SGI neurons need not be preceded by activation of overlying SGS neurons [20]. The existence of an interlaminar connection has been demonstrated anatomically by Moschovakis and his colleagues [21,22], who used intracellular staining techniques in the squirrel monkey; and in the hamster by Rhoades and his co-workers [23–25]. These results have been confirmed in other animal species, also through using anatomical tracing techniques [26,27]. Finally, Lopez-Barneo and Llinás [28], and later, Lo and Mize [29], used an intracellular staining technique in slice preparations to study the properties of neurons in the optic layer, or neurons in the deeper layers with dendritic arborisations projected into the sSC, that might mediate visual input to the dSC neurons.

Even though many of these authors suggested that an interlaminar connection might be involved in executing express saccades, the answer to the question ‘how is the synaptic transmission through the pathway gated?’ has remained unsolved until recently.

Anatomical and physiological evidence for the interlaminar connection

The synaptic mechanism and modulation of the interlaminar connection have been studied by my group [32] and more recently by Hall and colleagues [30,31], using whole-cell patch-clamp recording and intracellular staining techniques in slice preparations of the SC, which offer powerful approaches for studying the dynamic properties of local brain circuits.

Figure 2a summarises the design of our experiments in slices from rat [32]. Stimulating electrodes were placed in the SGS and/or in the optic tract (OT) near the lateral border of the optic layer (SO), where the OT comprises a bundle of fibres. The responses of SGS, SO and SGI neurons evoked by stimulation at these sites were recorded in a whole-cell configuration. A single electrical stimulus to the OT induced short-latency monosynaptic EPSPs (Figure 2b, 2 and 3) in narrow-field vertical cells of the SGS, which issued axonal projections extensively to the SO. In wide-field vertical cells in the SO, which project to the SGI (Figure 2b, 1), the same OT stimulus induced monosynaptic EPSPs (judged from short-latency and fixed onset) but their much slower rising phase suggested that the OT makes synapses on the dendrites that are remote to the soma (Figure 2c, 2 and 3).

In both cases, the EPSPs were enhanced by application of bicuculline (Bic), an antagonist of the γ-aminobutyric acid type A (GABA_A) receptor (Figure 2b, 4, and Figure 2c, 4), were suppressed partially by application of 2-amino-5-phosphonovalerate (AP5), an N-methyl-D-aspartic acid (NMDA) receptor antagonist (Figure 2b, 5, and Figure 2c, 5) and were suppressed completely by additional application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), an antagonist of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor (Figure 2b, 6, and Figure 2c, 6). These findings demonstrated that both SGS and SO neurons receive glutamatergic monosynaptic excitation from the OT.

By contrast, in the SGI neurons (Figure 2d, 1), as judged by their longer latency and fluctuating latencies, the EPSPs evoked after stimulation of the OT were disynaptic or oligosynaptic (Figure 2d, 2 and 3). These responses were enhanced markedly by application of Bic (Figure 2d, 4); the bursting spike discharges evoked in the SGI neurons could be superimposed on the long-lasting depolarisation — the duration of which could exceed 1 s in response to a single OT stimulation. The long-lasting depolarisation and bursting spike discharges were evoked in an all-or-none manner at threshold stimulus intensities (Figure 2d, 5). Stimulation
of the SGS induced monosynaptic EPSPs in SGI neurons (Figure 2e, 1), which were again amplified into bursting spike discharges that could be superimposed on the long-lasting depolarisation after application of Bic (Figure 2e, 2). These results confirm the existence of an excitatory pathway from the OT to SGI neurons, which is presumably mediated by SGS or SO neurons; this pathway has been also demonstrated by Lee et al. [30] using whole-cell recording in SC slices from the tree shrew.

In these in vitro experiments, we also explored neurotransmitters that might modulate the threshold for generating bursts in response to the synaptic drive in SGI neurons. We considered acetylcholine (ACh), which is released by pathways to the SGI from the pedunculopontine and laterodorsal tegmental nuclei [33–36], as a candidate for such a modulatory transmitter. In experiments carried out on rat slices, we found that application of ACh primarily activated nicotinic ACh receptors on SGI neurons and caused their depolarisation (T Isa and Y Saito, unpublished data).

On the basis of those observations, we microinjected nicotine locally into the SGI of awake monkeys while they were performing visually guided saccade tasks [37]. Nicotine induced a stepwise reduction in SRTs from the range of regular saccades (150–160 ms) to that of express saccades (about 100 ms; Figure 3). Notably, the SRTs showed a clear bimodal distribution after nicotine injection started and even during recovery from it. Few saccades of intermediate SRTs were elicited (Figure 3). Increasing the dose of nicotine caused no further reduction in SRTs. Our results suggest that express saccades may be produced by local increases in the excitability of dSC neurons. They also suggest that these nicotine-induced express saccades are executed by signal transmission through the neural pathway with the shortest transmission time, which is probably located within the SC.

The interlaminar projection of the superficial to the deeper tectal layers might provide such a pathway. When the signal flow through the interlaminar connection is enabled, for example, the visual signals that reach the sSC might be transformed into the burst activities of the dSC neurons, which comprise motor commands to trigger the downstream saccade generator circuits.

**Mechanisms for generating burst activities in the deeper layer neurons**

In principle, the saccade-related burst of SC neurons could be derived from two alternative mechanisms: first, the burst might arise from the properties of the intrinsic circuits of the SC; second, the type of input signal, such as that from the frontal eye fields, might impose the pattern of excitation [38].

The bursting responses of neurons could be mediated by several possible mechanisms: (1) low-threshold spikes causing transient bursting spike activity in burst spiking neurons [39]; (2) Ca²⁺-dependent plateau potentials [40]; (3) a hyperpolarisation-activated current such as that shown to cause rhythmic bursting activity in other regions of the central nervous system and that observed in wide-field vertical cells of the SGI [29,41,42]; (4) intrinsic membrane properties consistent with a nonlinear frequency–input relationship such as that demonstrated in feline SC neurons by Grantyn et al. [43,44]; and (5) NMDA-type glutamate-receptor-mediated synaptic transmission.

To explore this issue, it is essential to know the intrinsic firing properties of individual identified tectofugal cells, which would be responsible for the first four possibilities above. We have examined these properties previously [42] and more recently by identifying the crossed tectoreticular SGI cells using retrograde labelling with a fluorescent tracer (Y Saito Y and T Isa, unpublished data). In these studies, we found that the crossed tectoreticular neurons show regular spiking properties and a quasilinear frequency–input relationship. In addition, only a small population of tectofugal neurons showed burst spiking or plateau potentials.
Furthermore, a hyperpolarisation-activated current could be found only in wide-field vertical cells, which project primarily to the lateral pulvinar and to SO and SGI.

By contrast, the bursts emitted by SGI neurons in response to stimulation of the SGS in the presence of Bic were suppressed by application of 50 μM AP5, which indicates that the bursts depend on the activation of NMDA-type glutamate receptors (Figure 2e, 3). It is well known that NMDA receptors have a J-shaped current–voltage relationship [45]. In the presence of an Mg2+ block, the NMDA-type glutamate receptors admit inward currents only when the cell is depolarised sufficiently. Once the membrane potential exceeds the threshold for sufficient activation of the NMDA receptors, a regenerative process ensues that further enhances their depolarisation. Such a nonlinear activation of NMDA receptors could account for the all-or-none characteristics of the bursts of the SGI neurons.

As described above, the saccade-related bursts might not arise through the intrinsic properties of individual SGI neurons but could be caused by NMDA-type receptor-mediated synaptic transmission. But even though the NMDA-type receptor-mediated EPSPs show a long time course, this cannot explain the long-lasting depolarisation observed in the SGI neurons, which often exceeds 1 s. Presaccadic burst neurons of the squirrel monkey SC are known to have recurrent collaterals that ramify in the neighbourhood of the somata [21,22]. We have also observed these local collaterals in the SGI [32,42] (Figure 2d, 1). These observations may suggest that the recurrent excitatory network would function as the basis for the long lasting depolarization in addition to the NMDA receptor-mediated synaptic transmission.

Pettit et al. [46] used a photostimulation technique to induce a local release of free glutamate from a caged glutamate compound and found that the local SC circuits contain mechanisms to induce bursting in SGI neurons. They proposed that there is lateral excitatory interaction (or disinhibition) among SGI neurons. In support of a role for disinhibition in generating these bursts, Pettit et al. [46] showed that although Bic is not necessary for SGI neurons to emit bursts, their depolarisation is far more vigorous and long lasting if GABA_A receptor-mediated inhibition is reduced by this antagonist. The morphology and application of the lateral excitatory interaction in models of the SC has been discussed recently by Bozis and Moschovakis [47].

### Disinhibition from what?

As described above, disinhibition from GABA_A receptor-mediated inhibition unmask signals transmission through the interlaminar connection and the bursting activity of dSC neurons. This naturally raises the question: which GABAergic system is involved in suppressing the interlaminar pathway and the bursting response of dSC neurons?

In the cat and monkey, substantia nigra pars reticulata efferents have been shown to contact [48] and to inhibit dSC neurons through GABA_A receptor synapses [21,49]. A similar pattern of inhibition may be mediated by neurons in the rostral pole of the SC (fixation neurons) [50]. Both classes of cells are tonically active while the animals are fixating the eyeball and pause firing just before saccade onset in monkeys, thereby releasing dSC neurons from tonic GABA_A-receptor-mediated inhibition before the onset of saccades [50–52]. Thus, disinhibition actually occurs in dSC neurons before execution of saccades in vivo, although the magnitude of disinhibition may vary depending on the behavioural status and might be only partial in comparison to what we have observed during application of 10 μM Bic to the slices.

In the slice preparation, Bic was needed to unmask the interlaminar pathway and the bursts of SGI neurons even after removal of the substantia nigra pars reticulata from the slices. Thus, under the current experimental preparation, it is more likely that electrical stimulation of the OT or the SGS evoked inhibitory postsynaptic potentials (IPSPs) in addition to the EPSPs through GABAergic interneurons in the SC. Such stimulus-linked IPSPs might suppress the prolongation of excitatory inputs to the SGI neurons. It is well known that the SC contains many GABAergic neurons. In particular, several GABAergic neurons are located in the sSC [53]. At present, it is not clear which group of GABAergic neurons, located in either the sSC or the dSC, are involved in such an inhibition linked to the OT or SGS stimulation.

In this regard, Meredith and Ramoa [54] have shown recently that stimulation of the rostral part of the SC induces inhibition in caudal SC neurons through local inhibitory interneurons because the inhibition disappears after application of glutamate receptor antagonists. In addition, they showed that this inhibition remains after an incision is made to the slice to remove the effects mediated by the sSC. On the basis of these observations, Meredith and Ramoa [54] have concluded that GABAergic neurons in the dSC are crucial for mediating the inhibition between the remote regions in the SC [55]. If the inhibitory interneurons in the dSC are involved in the tonic suppression of saccade-related burst neurons by fixation neurons, then their role in regulating the activation of collicular local circuits should become more and more apparent in future studies.

### Arguments on the ‘moving hill’ hypothesis

Guitton et al. [56] proposed that the dynamic motor error of saccades are coded in the spatial pattern of population activity in the SC. From their experiments in cat, they suggested that saccades are initiated by a hill of activity at the caudal site appropriate for a particular saccade and that, as the saccade evolves and the motor error decreases, the hill moves rostrally across successive SC sites responsible for saccades of smaller amplitudes [57]. Subsequently, Munoz and Wurtz [58] showed that the moving hill is
represented by a subpopulation of dSC neurons with prelude activity in monkeys.

This ‘moving hill’ hypothesis has attracted attention both from physiological and computational point of views and in the past decade has been a principal issue in research into intrinsic processing in the mammalian SC. Support for the moving hill hypothesis has been obtained recently through comparison of the activation sequences of simultaneously recorded neuron pairs of dSC neurons at population level [59]. By contrast, Moschovakis and co-workers [60••], by imaging the active cell population of SC cells using 14C-labelled deoxyglucose, and Fuchs and colleagues [61•], by making single-unit recordings in the monkey SC, have found evidence negating this hypothesis.

Although it has been shown that the lateral excitatory connection is potent in the dSC [32,46,49], a polarised distribution of axonal projections or dendritic protrusions that might substantiate the asymmetric spreading of excitation for the ‘moving hill’ has not been described in previous studies on the axodendritic morphology of dSC neurons [21,22,42,49]. This may be because previous studies were not aimed at testing the feasibility of the ‘moving hill’ hypothesis and so morphological evidence in support of the polarised spread of excitation was simply missed. Alternatively, even if the ‘moving hill’ does exist, then it might be brought about by extrinsic inputs. As well as the feasibility of the ‘moving hill’ hypothesis itself, the structural background of ‘the moving hill’ remains an open issue.

**Signal processing in the superficial layers**

As discussed early on by Edwards [19], the cytoarchitecture of the sSC is very different to that of the dSC, which displays histological characteristics of the reticular core. The sSC has a cytoarchitecture characteristic of a sensory structure, and the morphological classification of its cell subtypes was established in an earlier study or the Golgi [62].

Although I have not discussed it here in depth, the electrophysiological properties of the intrinsic firing pattern of individual cells and synaptic thalamocortical transmission have been studied by whole-cell recordings from recognised cell subtypes in combination with intracellular staining techniques in slice preparations [29,63••,64•–67•]. In addition, neuropharmacological aspects of characteristic visual sensory processing, such as surround inhibition and adaptation, have been determined from *in vivo* anaesthetised preparations using extracellular unit recording coupled with iontophoretic applications of agents [68].

Although not all of the details of the data obtained in these two different experimental preparations seem to concur [67•], a combination of different approaches to experimental models will surely deepen our understanding of signal processing in this structure.

**Conclusions and future directions**

As described above, we are finally obtaining a positive answer to the long-standing debate on the existence of the interlaminar connection in the SC, although direct evidence in primates has not been obtained as yet. Interest should now shift to modulation of the signal transmission through this connection and its functional implication. In this context, GABAergic interneurons in the SC seem to be crucial in modulating the activation level of the SC local circuits. However, recently developed transgenic techniques raise the possibility of labelling the neurons of specific transmitter subtypes through expression of enhanced green fluorescent protein. Using such transgenic animals might give us an opportunity to record from identified GABAergic neurons and to study the specific neuronal mechanism that regulates GABAergic transmission in the SC.

The SC is an excellent system to study in slice preparations, because the input–output relationships of its afferent and efferent pathways and the information encoded by individual neurons have been well defined in behaving animals. As exemplified in this review, slices obtained from rodents have been used to study in detail the fundamental structure of the SC local circuits — that is, the synaptic relationships between the neurons that the SC contains and the mechanisms that are responsible for their modulation. The information obtained from these *in vitro* studies has been used subsequently to design *in vivo* experiments in behaving monkeys. Combining these two experimental approaches may open new avenues for understanding how the cognitive behaviour of the awake, behaving animal is influenced by the dynamic regulation of signal transmission in local SC circuits. Such study will lead to a deeper understanding of the role of neuronal activities in the neural structures that send modulatory inputs to the SC, such as the pedunculopontine tegmental nucleus that sends cholinergic projections to the SGI, as we have recently identified in behaving monkeys [72•].

**Update**

Since this article was submitted, a novel article was published on the organisation of GABAergic neurons in the SC. The article particularly focused on their expression patterns of various chemical markers [75•].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest  
** of outstanding interest


and midbrain dopaminergic systems. Neurons related to saccade preparation and execution, reward and performance level of the task are identified. A combination of responses related to saccade execution, reward delivery and task performance is observed. It is concluded from the multimodality of responses in PPTN neurons that the PPTN may act as an integrative interface between the various signals required for performing purposive behaviours.


The authors investigated colocalization of several chemical markers such as calbindin, calebrinin, parvalbumin, and neuronal nitric oxidase synthase with GABAergic markers in the ferret's SC neurons and were able to show that there is significant heterogeneity of GABAergic neurons in the SC.