PRIMATE SUPERIOR COLLICULUS NEURONS ACTIVE IN A GOAL-
DIRECTED ARM MOVEMENT TASK INVOLVING HAND-TARGET
CONTACT AND EXTERNAL TASK PERTURBATION

by

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Statement

I certify herewith that the dissertation at hand was completed and written independently and without outside assistance. The "Guidelines for Good Scientific Practice" according to § 9, Sec. 3 were adhered to. This work has never been submitted in this or a similar form at this or any other domestic or foreign institution of higher learning as a dissertation.

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## METHODS

1
ABSTRACT

Current neuroscience often approaches the nervous system from the sensorimotor transformation perspective whereby changes in the body and environment are detected by senses and this sensation together with the current state of the body determines animal’s movements. Previous research approach has often been influenced by designated borders between processing of sensory input and motor output. Here, we report novel types of neuronal activity within the primate midbrain structure the Superior Colliculus (SC) that illustrate the interdependence between sensation and movement. The SC has been mainly investigated within the context of its role in gaze orienting. In the focus of this study are SC neuronal populations activated for a hand-target contact within goal directed arm movements. We recorded activity of individual SC neurons in a rhesus monkey subject trained to press illuminated buttons on the working panel while maintaining visual fixation. To initiate a task trial the subject was required to put a hand on the start position in a horizontal plane and to fixate a dot of light that appeared shortly after on the vertical working panel. One button on the panel was then lit red followed by a change to green, which was a ‘go’ signal for the subject to press the button. After pressing and maintaining the contact, the subject finished the trial by returning the hand to the start position. During the whole trial visual fixation was required; this was introduced to assure that recorded neuronal activity was not due to eye movements. In the deep SC zone we found neurons activated for the hand-target contact. Their responses were typically weak or absent for a task-irrelevant somatosensory stimulation of the hand, however vigorous for a goal-directed contact. In addition, we found a subpopulation of neurons activated immediately before a contact with a target. To examine how these neuronal types respond in the presence of an external event
when sensation is not solely a result of a self-generated movement we introduced an unexpected, abrupt panel motion triggered by the contact in some trials. Majority of neurons expressed modulated activation in the presence of this perturbation. In addition, we found a new class of neurons that were activated exclusively during perturbed trials. Some of these neurons had unusually long latencies upon the perturbation. Two major control tasks have been introduced to test whether the last neuronal population has been activated solely by somatosensory disturbance of the contact. The first control task was the same as the standard one with the exception that the monkey did not touch the panel. The second control task involved identical sound that was audible during perturbation, without the panel motion and the contact. Both paradigms elicited responses in tested neurons. These responses appeared to be event-specific because visual and auditory stimuli that were not identical to those produced during perturbation did not elicit a response. If the neuronal activity emerging before the contact relates to the anticipation of the sensory consequence and if the neuronal activity emerging exclusively upon perturbation relates to the discrepancy between the expected and the experienced sensation then the reported neuronal classes may together participate in networks that differentiate self-induced vs. external events.
INTRODUCTION

Contact of the hand with the target presents a critical part of a goal-directed arm movement. It is often a goal by itself as when one touches the shoulder of a neighbor or may present a mean to acquire a goal as when opening the door. The event of contact comprises phenomena that may pose challenges for neuroscience. Imagine yourself approaching a door that you want to open. In the final stage of this endeavor the hand moves towards the door handle and the contact ultimately occurs. This event causes a specific sensation informing that the hand arrived on the target. This sensation is not surprising for our brain since it is self-induced. If, however, somebody else at the same time unexpectedly pushes the door from the other side, this induces one additional sensory input that could not be anticipated. This input would inform that something externally happened. The phenomenon of the contact illustrates the interplay between sensation and movement challenging artificially designated borders between processing of sensory input and motor output. Investigation of neuronal signals occurring during hand-target contact within goal-directed arm movements is in the focus of this thesis.

Specifically, activity of individual neurons was extracellularly recorded from the midbrain Superior Colliculus (SC) of a rhesus macaque monkey while he performed a task involving goal directed arm movements. Here, an overview of the primate SC is provided, together with references to other species. If not stated otherwise, the text refers to the rhesus monkey.

Primate Superior Colliculus

The SC is a bipartite laminated structure symmetrically located on either side of the midline on the dorsum of the midbrain. It is generally agreed that the SC is composed of seven main layers (e.g. May 2006): the zonal layer (*stratum zonale,*
SZ), the superficial grey layer (stratum griseum superficiale, SGS), the optical layer (stratum opticum, SO), the intermediate grey layer (stratum griseum intermediale, SGI), the intermediate white layer (stratum album intermediale, SAI), the deep grey layer (stratum griseum profundum, SGP), and the deep white layer (stratum album profundum, SAP).

The first three layers compose the superficial zone of the SC, which appears to be exclusively responsive to visual stimuli; neurons of this zone are organized into retinotopic map and connected to other visual-processing areas (Cynader & Berman 1972; Goldberg & Wurtz 1972). They respond to visual stimuli presented in a specific part of visual space relative to the point of current fixation (Cynader & Berman 1972; Goldberg & Wurtz 1972). This part of the visual field is denominated as a neuron’s visual response field or receptive field (RF). Neurons responding to one part of the visual field are situated adjacently and all together form a retinotopic map: neurons in the rostral pole respond to stimuli presented close to the point of current fixation, and as one moves towards the caudal pole neurons respond to stimuli farther away from the fixation point; in addition, neurons in the lateral SC respond to stimuli presented below and in the medial SC above the horizon (Figure 1).

The four deeper SC layers appear to represent an integration site for a variety of neuronal signals from different brain areas (Hall & Moschovakis 2004). The most studied group of neurons are those that exhibit activity in advance of and during eye movements to a specific part of the visual space, which defines their response field (Dorris et al 1997; Munoz & Wurtz 1995a; b; Schiller & Stryker 1972; Sparks 1975; Wurtz & Goldberg 1972). These response fields (also known as motor fields) are organized in a topographical map appearing to be congruent with the map in the superficial layers; neurons in the rostral pole are active for eye movements directed to locations close to the point of current fixation, and as one moves towards the
**Figure 1.** Retinotopic Map of the SC

The upper panel represents the cross section of the SC surface with a corresponding retinotopic map (modified after the original laboratory map created by Hoffmann KP, anesthetized preparation): Solid parallels comprise points corresponding to equal distance from the fixation point in degrees of visual angle with values on the left side. Note overrepresentation of the foveal region. Dashed meridians correspond to steps of 10 degrees of angle from the horizon whereby the horizon is along the SC line represented with a solid line.

The lower panel represents a visual field in front of the monkey: The centre represents a fixation point, concentric circles correspond to the visual angle in steps of 10 degrees, and numbers on the outer circle present angle in the visual field.

The red point illustrates correspondence between the recording site in the SC and the receptive field coordinates in the visual filed.
caudal pole neurons are active for eye movements directed to locations farther away from the fixation point; in addition, neurons in the lateral SC are active for eye movements directed to locations below and in the medial SC above the horizon (Hall & Moschovakis 2004). In addition, the intermediate SC layers comprise fixation neurons that are active during visual fixation, and pause immediately before and during the eye movement, to resume the activity at the time of saccade termination (Munoz & Wurtz 1993a; b). Electrical microstimulation of the deeper SC layers produces eye movements according to the described topographical organization (e.g. Robinson 1972). However, in the majority of studies the head of the monkey was restrained, thus only eye movements could be studied. Studies employing head unrestrained preparation provided a more general insight into the role of the primate SC suggesting that e.g. electrical microstimulation of the deeper SC layers produces combined eye and head movements in order to redirect the gaze as a whole (Freedman et al 1996; Sparks 1999; Sparks et al 2001).

Furthermore, the deep and intermediate SC layers comprise neurons responding to auditory (Jay & Sparks 1984; 1987), visual (e.g. Cynader & Berman 1972), somatosensory stimuli (Groh & Sparks 1996), and multimodal neurons responding to the combination of stimuli from different modalities while having their response often higher for the combination than for a sum of responses to unimodal stimuli alone (Stein & Stanford 2008). All of these neuronal classes are mostly discussed as subserving a sensorimotor transformation system in the SC that integrates sensory and cognitive inputs and, and via mechanisms of competition produces a motor output signal that ultimately results in a gaze-orienting movement towards the salient event (Hall & Moschovakis 2004; Munoz 2002; Munoz & Fecteau 2002). The receptive fields of auditory, visual, and somatosensory signals are mostly aligned in space (e.g. an auditory stimulus occurring at one location in space will
activate neurons in the same zone of the SC map as a visual stimulus occurring at
the same location; in addition, if a neuron responds to both auditory and visual stimuli
its auditory and receptive field overlap) which is thought to support integrating signals
originating from a single event leading to a motor program that orients the gaze
towards this one spatial location (for a review see Stein & Stanford 2008).

Inactivation of the deep and intermediate SC layers appears to disrupt target
selection in the affected zone, without direct effect on eye movements per se
(McPeek & Keller 2004). Namely, upon reversible inactivation by muscimol, eye
movements are misdirected to distracter stimuli, however normal if only one target is
in the visual field (McPeek & Keller 2004). Thus, the SC appears to be functionally
involved in target selection. Furthermore, the deep and intermediate SC layers seem
to be critical for allocating covert attention, which will be discussed below (Lovejoy &
Krauzlis 2010).

**Anatomy of the SC**

Here, an overview of the primate SC anatomy would be provided from the
perspective of input and output neuronal projections.

**Inputs to the Superficial SC Layers**

The SC superficial zone receives direct retinal projections, which form the
retinotectal pathway (Leventhal et al 1981; Marrocco & Li 1977; Mize et al 1991;
Perry & Cowey 1984; Pollack & Hickey 1979; Rodieck & Watanabe 1993). This
pathway appears less pronounced in monkey since only 10% of retinal axons project
to the SC (Perry & Cowey 1984; Pollack & Hickey 1979) as compared to 50% in cat
(Wassle & Illing 1980), and 70% in mouse (Hofbauer & Drager 1985). Retinotectal
projections are thought to be primarily glutamatergic based on evidence in cat (Mize
& Butler 1996); in monkey *Cebus apella* there is evidence about only small proportion
(less than 3%) of GABAergic (utilizing gamma aminobutyric acid as a neurotransmitter) retinotectal ganglion cells (da Costa et al 1997).

In addition, the SC superficial layers receive projections from the visual cortex including areas 17, 18, and 19 (Abel et al 1997; Finlay et al 1976; Fries 1984; Lia & Olavarria 1996; Lock et al 2003; Lui et al 1995; Marrocco 1978; Marrocco & Li 1977; Mize et al 1991; Tiges & Tiges 1981), midtemporal area (Fries 1984; 1985; Lock et al 2003), and the superior temporal sulcus (Lui et al 1995; Webster et al 1993). It is believed that these projections are glutamatergic based on studies in cat (Binns & Salt 2000; Mize & Butler 1996). The role of the projections from the occipital visual cortex to the superficial layers in rhesus macaque monkey is not clear. Reversible cooling of the occipital visual cortex produced almost no effect on visual responses of neurons in the superficial layers, whereas visual responses of neurons in the deep layers were reduced or abolished (Schiller et al 1979).

Furthermore, there are strong reciprocal connections between the superficial SC layers and the parabigeminal nucleus (Baizer et al 1991; Harting et al 1980), whereby the inputs from the parabigeminal nucleus mainly terminate in the SGS (Baizer et al 1991) and between the superficial SC layers and pretectum (May 2006), whereby pretectal inputs originate mainly from the nucleus of the optic tract, as well as the posterior pretectal nucleus (May 2006) and terminate primarily in SGS, and in a lesser extent in SO (Büttner-Ennever et al 1996; May 2006).

**Outputs of the Superficial SC Layers**

The superficial SC layers project to the pretectum, primarily to the nucleus of the optic tract and the posterior pretectal nucleus, with much less terminals in the olivary pretectal nucleus (May 2006). Furthermore, they project to the parabigeminal nucleus as a part of the mentioned strong reciprocal connection between the SC and this nucleus (Harting et al 1980). In addition, the lateral geniculate nucleus (LGN) is
one of major targets of the superficial SC layers output and these projections form the tectogeniculate pathway (Harting et al 1991). These include neurons of the SGS whose axons target the dorsal LGN (Wilson et al 1995) as well as the lateral LGN in its layer that is adjacent to the optic tract (Harting et al 1980). Another target in the thalamus presents the pulvinar, and these output projections originate both from SGS and SO (Benevento & Standage 1983; Huerta & Harting 1983). It appears that these terminate predominantly in ipsilateral inferior pulvinar (Harting et al 1980; Stepniewska et al 2000), and in a less extent in lateral (Benevento & Standage 1983; Harting et al 1980), medial (Benevento & Standage 1983) and dorsal pulvinar (Harting et al 1980). However, visual responses in the inferior pulvinar appear to rely mainly on the cortex and not the SC; cortical lesions abolished visual responses of majority of pulvinar neurons, while SC lesions in comparison produced rather subtle effect, primarily increasing latency of visual responses in cells that are not sensitive to stimulus orientation (Bender 1983).

**Inputs to the Deep and Intermediate SC Layers**

Physiological as well as anatomical evidence about SC connections with the rest of the brain suggest that neurons in the SC deep and intermediate layers integrate signals arriving from multiple brain structures as the retina (Beckstead & Frankfurter 1983), the visual cortex (Lui et al 1995), the frontal eye field (Fries 1984; Hanes & Wurtz 2001; Segraves & Goldberg 1987; Sommer & Wurtz 2001), the lateral intraparietal area (Paré & Wurtz 1997; 2001), the substantia nigra pars reticulata (Hikosaka & Wurtz 1983b; 1985), the dorsolateral prefrontal cortex (Fries 1984; Johnston & Everling 2006; Selemon & Goldman-Rakic 1988; Tanila et al 1993), the cerebellum (Gonzalo-Ruiz & Leichnetz 1990; Gonzalo-Ruiz et al 1988; May 2006; May et al 1990), and the premotor and motor cortices (Fries 1985).
Direct retinal projections appear to terminate in the SGI (Beckstead & Frankfurter 1983). The input from the visual cortex is suggested to originate mainly in the area 19 and terminate in the SGI as well (Lui et al 1995).

The frontal eye field, cortical structure intimately involved in eye movement processing and visual attention (Amiez & Petrides 2009; Tehovnik et al 2000) projects both to the SC (Segraves & Goldberg 1987) and directly to the brainstem oculomotor circuits that issue a final command to move the eyes (Sparks 2002; Sparks et al 2001). Antidromic electrical microstimulation, which required the lowest current threshold in the intermediate SC layers, demonstrated direct projections from the frontal eye field to the SC, suggesting cortical layer V, as a main source (Segraves & Goldberg 1987). In addition, inactivation of the SC proposed that the frontal eye field predominantly through the pathway via the SC influences oculomotor behaviour (Hanes & Wurtz 2001), recognizing again the pivotal role of the SC in the oculomotor circuitry.

Input from the lateral intraparietal area to the intermediate SC layers is physiologically evidenced by antidromic electrical microstimulation in the intermediate SC layers and this input seems to contain both visual and oculomotor signals (Paré & Wurtz 1997). The role of the lateral intraparietal area has been debated, having confronted views about its predominantly sensory (e.g. attentional) (Colby & Goldberg 1999) or motor (e.g. in forming motor intentions to move the eyes) (Andersen & Buneo 2002). It appears that the lateral intraparietal area is involved in both, certainly being an important structure within the eye movement circuitry (Bisley & Goldberg 2010; Goldberg et al 2006). As regards connection with the SC, neurons in the lateral intraparietal area projecting to the SC appear to discharge less vigorously for an eye movement than the SC eye movement neurons, and their activity appear to rely more on the visual input i.e. presence of the visual target, than
of the SC neurons suggesting closer relationship of the SC neurons to eye movements per se (Paré & Wurtz 2001).

The deep and intermediate SC layers receive inhibitory projections from the substantia nigra pars reticulata (Beckstead & Frankfurter 1982; Jayaraman et al 1977), and these projections have strong ipsilateral dominance (Beckstead & Frankfurter 1982; May 2006). In addition, antidromic stimulation in the SC confirmed existence of such direct projections, suggesting that axons from the substantia nigra pars reticulata enter deep layers of the SC and arborize mostly in the intermediate layers where eye movement neurons are located (Hikosaka & Wurtz 1983b). These projections play a role in the well studied eye movement circuitry and may serve as a model for a general gating function of the basal ganglia in motor control (Grillner et al 2008). Namely, eye movement related neurons in the substantia nigra pars reticulata are tonically active and importantly pause for an eye movement (Hikosaka & Wurtz 1983a). This is thought to present a critical part of the eye movements control circuit that involves pathway from the dorsolateral prefrontal cortex that excites GABAergic neurons in the caudate nucleus, which in turn inhibit GABAergic neurons in the substantia nigra pars reticulata; thus, activation of the dorsolateral prefrontal cortex neurons will via two inhibitory synapses result in a disinhibition of the SC by the substantia nigra pars reticulata and allow an eye movement (for a review see: (Hikosaka et al 2000; Munoz 2002).

Input from the dorsolateral prefrontal cortex evidenced by antidromic stimulation in the intermediate SC layers seems to carry task dependent signals (Johnston & Everling 2006). Specifically, some neurons in the dorsolateral prefrontal cortex are activated when monkeys had to make eye movements away from the visual stimulus, which is considered to require inhibition of a reflexive eye movement towards the stimulus (Johnston & Everling 2006; Munoz & Everling 2004). This
suggests that the intermediate SC layers may receive inhibitory signals for unwanted eye movements partly from the dorsolateral prefrontal cortex. This signal, however, may act on inhibitory interneurons or on fixation SC neurons since cortical projections generally are believed to be glutamatergic (e.g. Bear et al 2001)).

There is a strong interconnection between the SC and the cerebellum: SGI receives contralateral input from the posterior interposed nucleus and adjacent dentate nuclei; in addition, SGI receives input from the fastigial nucleus (bilateral projection with contralateral predominance) and in this case the main target zone is the rostral pole of the SC (Gonzalo-Ruiz & Leichnetz 1990; Gonzalo-Ruiz et al 1988; May 2006; May et al 1990). The relationship between the SC and the cerebellum has mainly been considered in the context of the oculomotor function inspiring modeling efforts, some of which tried to emphasize the role of the cerebellum in assuring the accuracy of an eye movement, and the role of the SC in determining the target and providing a general direction of the movement (Lefevre et al 1998; Quaia et al 1999).

Finally, anatomical evidence suggests influence of motor and premotor cortices over the SC (Fries 1984; 1985). Projections from these cortices appear to have topographical organization in some extent with cells from putative finger, hand, arm and shoulder representation projecting to the rostrolateral SC zone, and cells from putative arm, and trunk representation projecting to the caudal SC zone (Fries 1985). Neuronal recordings revealed the existence of neurons that are active in advance and during arm movements located within the intermediate and deep SC layers (Kutz et al 1997; Lünenburger et al 2001; Werner 1993; Werner et al 1997a; Werner et al 1997b). This neuronal class will be discussed separately.

In addition to discussed connections, the seminal study of Fries revealed projections from: posterior parietal cortex (area 7) with a highest concentration on the posterior bank of the intraparietal fissure; inferotemporal cortex (areas 20 and 21);
auditory cortex (area 22); somatosensory representation SII (anterior bank of sylvian fissure, area 2), whereby the projection region appears to correspond to fingers, and hand representation; and the upper insular cortex (area 14) (Fries 1984).

**Outputs of the Deep and Intermediate SC Layers**

The SC influences structures that send projections to the SC as if forming functional loops. One of main routes is via the thalamus, whereby the SC projections terminate in different thalamic nuclei (Harting et al 1980). The frontal eye field, and in addition, the supplementary eye field, appear to be targeted via the central lateral nucleus and the adjacent paralamellar part of the medial dorsal nucleus predominantly by the ipsilateral SGI (Huerta et al 1986; Lynch et al 1994). Disynaptic pathway from the SO and SGI via the lateral part of the mediodorsal nucleus was directly evidenced by retrograde transport of the herpes simplex virus type I injected in the frontal eye field (Lynch et al 1994). Signal flow from the intermediate and deep SC layers to the frontal eye field via mediodorsal thalamus is physiologically evidenced by combined antidromic and orthodromic stimulation (Sommer & Wurtz 2004; 2006). Specifically, neurons in the mediodorsal thalamus were identified that can be activated by the orthodromic electrical microstimulation in the intermediate SC layers and by the antidromic electrical microstimulation in the frontal eye field. This pathway is suggested to carry a corollary discharge signal from the SC to the frontal eye field, about the upcoming eye movement (Sommer & Wurtz 2006).

The descending output from intermediate and deep SC layers provides, amongst other, a single saccadic command to the nuclei of ocular motor neurons in the brainstem, which directly innervate extraocular eye muscles providing the final control signal for the eye movement (for a review see Fuchs et al. 1985, Scudder et al. 2002, Sparks 2002).
SC and Goal-Directed Locomotion

In non mammals, the optic tectum, a non mammalian homologue of the SC, appears to have capacity of governing locomotion (Grillner et al 2008; Saitoh et al 2007). Electrical microstimulation in the optic tectum of the lamprey causes eye movements, at longer stimulation duration causes body orientation movements correlated with the eye movements, and at even longer duration locomotor movements (Saitoh et al 2007).

Based on anatomical evidence in monkey about the deep SC layers projections to the cuneiform nucleus (Harting 1977), and the observation that electrical microstimulation in the monkey (Macaca Fascicularis) cuneiform nucleus induces stepping locomotion (Eidelberg et al 1981) one may hypothesize that SC could influence locomotion. Such SC role has not been systematically examined in monkey.

However, in rat it has been suggested that the SC can be involved in goal-directed locomotor behaviour, specifically in selecting towards which spatial location rats move (Felsen & Mainen 2008). Felsen and Meinen recorded the SC neuronal activity in rats engaged in an odour-discrimination task whereby they moved to one of two locations based on the odour instruction given at the central port. They found populations of SC neurons in intermediate and deep layers that were active during locomotion towards a specific spatial goal, in advance of such motor program, and while rats lingered at the achieved location. In addition, after reversible inactivation of one SC with muscimol (muscimol injection was targeted to intermediate and deep layers), rats were biased to acquire the location on the ipsilateral side. Importantly, although animals had instruction to move towards a specific location on one side of the central port, inactivation of the SC concerned with that side (i.e. contralateral SC) caused them to move often towards the location on the other side. Therefore, the rat
SC appears to contain signals that govern goal-directed behaviour. In addition, neurons with a build-up activity during locomotion towards a switch which had to be pressed with the forepaw to receive food have been observed in the SC of the cat (Hoffmann KP, personal communication).

Together, these observations have important implications, suggesting a more general function of the SC in a goal-directed behaviour.

SC Connections with the Dopamine Brain System

There is both anatomical and physiological evidence about direct SC connections with the dopamine system in the rat brain, specifically with the substantia nigra pars compacta (SNc) (Comoli et al 2003; Dommett et al 2005) and recent anatomical evidence about such connections in monkey (May et al 2009). It appears that in the rat the SC provides a major sensory input to the dopamine neurons in the SNc (Comoli et al 2003; Dommett et al 2005). This has important implications on the understanding general function of the dopamine system. Namely, it has been proposed that dopamine neurons signal reward prediction errors such as upon a delivery of unexpected reward or upon an omission of the expected reward (Montague et al 1996; Montague et al 2004; Schultz 1998; 2002; 2006; Schultz & Dickinson 2000). However, this view has recently been challenged (Redgrave & Gurney 2006). Dopamine neurons respond to sensory stimuli associated with these events (e.g. to an image on the display that predicts a reward) at a very characteristic short latency, usually less than 100 ms (Schultz 1998). At a time of their firing, the visual system of the brain has still not discriminated the identity of such stimulus since this discrimination requires more detailed visual examination that occurs after gaze orientation to the stimulus. Thus, visual response of dopamine neurons has to rely on the visual signal that is available before gaze orienting and it can be communicated by the SC (Comoli et al 2003; Dommett et al 2005). This has critical
implication on the understanding of the dopamine system function. The dopamine signal according to Redgrave, Gurney, and colleagues may act on the striatum in synchrony with contextual and motor efference copy signals to reinforce a motor program that preceded an occurrence of a salient event (Redgrave & Gurney 2006). Importantly, the signal that the salient event occurred comes from the SC (Comoli et al 2003; Dommett et al 2005; Redgrave & Gurney 2006).

**SC Role in Attention**

Attention as one of the central phenomena of consciousness has been extensively investigated, primarily within visual neuroscience (for a review see Posner & Petersen 1990, Corbetta & Shulman 2002, Maunsell & Cook 2002, Moore 2003, Reynolds & Chelazzi 2004, Moore 2006, Knudsen 2007). It is usually classified into overt and covert attention (e.g. Moore 2006). When inspecting visual scene primates and other species make eye movements in order to bring images of objects of interest onto the fovea, a small central part of the retina, which contains the highest density of photoreceptors and has a disproportionately large part of the visual cortex devoted to processing its signal (Munoz 2002). In this way, a detailed perception of the object one is directly looking at is assured. These eye movements are landmarks of overt attention. On the other hand, attention can be allocated to one part of the visual scene while looking elsewhere and this is denominated as covert attention. In primates it often occurs when gaze orienting towards the part of visual scene is unwished as in some social circumstances (Moore et al 2003).

The SC as a pivotal structure for gaze orienting directly participates in allocating overt attention. A remaining question is whether the SC plays a role in allocating covert attention. Electrical microstimulation of the SC revealed that attending to one location necessarily induces eye movement preparation towards that location, as evidenced by deviation of microstimulation-evoked eye movements.
towards the attended location (Kustov & Robinson 1996). These results supported so-called premotor theory of attention stating that visual attention relies on eye movement premotor processing (e.g. Rizzolatti et al 1994). On the other hand, electrical microstimulation of the intermediate SC layers may improve visual perception in the stimulated zone of the peripheral visual field apparently without effects on eye movements per se (Müller et al 2005). Such evidence advocates a possible role of the SC in covert attention. Neuronal recordings within intermediate SC layers suggested that visuomotor neurons (neurons found mainly in the intermediate layers that are active both for a visual stimulus in their receptive field and for an eye movement to the same zone in the visual field) may present a part of a neuronal substrate for this SC role (Ignashchenkova et al 2004). Finally, inactivation of the SC appears to impair selection of targets for covert visual attention in the affected zone of the visual field (Lovejoy & Krauzlis 2010). Importantly, monkeys seemed to ignore information contained in the target placed in the affected zone only if it was accompanied by other meaningful targets in the visual field but not if presented alone (Lovejoy & Krauzlis 2010). This suggests that the SC is indeed functionally involved in covert attention and target selection processing.

**SC and Arm Movements**

Anatomical evidence about neuronal projections from the motor and premotor cortices suggested that the SC may participate in arm movements (Fries 1984; 1985). The SC has long been physiologically investigated however, its neuronal activity has been only relatively recently recorded during arm movement tasks (for a review see Lünenerburger et al 2001). These recordings revealed a novel classes of neurons discharging in advance of and/or during arm movements found in the intermediate and deep SC layers as well as in the underlying reticular formation (Kutz et al 1997; Werner 1993; Werner et al 1997a; Werner et al 1997b). Their activity
appeared to correlate maximally with rectified electromyograms of muscles within the shoulder girdle (Stuphorn et al 1999; Werner et al 1997a).

One group of those neurons (around 40%) showed gaze-dependency, having the level of activity being modulated by the position of the fixation point relative to the target (Stuphorn et al 2000). These neurons were found in the lower intermediate depth (around 2 mm) partially overlapping with the depth were eye movement SC neurons are typically located (around 1.5 mm). They had increased activity for specific eccentricities of the fixation point relative to the target of arm movements as if they had a response field. However, these response fields were not aligned with the established visual and oculomotor maps of the SC. In addition, those fields were located both in the ipsilateral and contralateral visual field as opposed to established visual and oculomotor maps that have response fields in the contralateral visual field. Finally, activity of these neurons was not modulated by a trajectory of the arm, as if they were not concerned with a specific muscle activation pattern of an arm movement but rather with the goal.

The majority of neurons (around 60%) had activity independent of gaze position i.e. they had a similar level of activation during arm movements to the target while the monkey visually fixated the target or different locations around the target. They tended to be located in the deep SC layers (around 4 mm) and to extend presumably to the underlying mesencephalic reticular formation. These neurons appeared to have more pronounced prelude activity as if reflecting motor preparation (Stuphorn et al 2000).

Finally, a recent study reported neurons within the intermediate and deep SC layers that were active during the contact with the target (Nagy et al 2006). Their activity seemed intriguing since it appeared in the context of a motor program i.e. when the monkey touched the target himself and almost not otherwise. They were
not activated during a reach phase of arm movements or for eye movements made to
the target. The nature of their activation remained unknown.

Scope of the Study

The research about eye movements established eye movement neuronal
circuitry as one of the best understood brain systems and recognized the SC as its
pivotal structure. However, emerging evidence suggests that the SC functions may
greatly exceed those currently estimated on the basis of this research. The SC’s
general role in goal-directed behaviour presents a particular challenge. Here we
investigate neuronal activity within the SC during goal-directed arm movements.
Specifically, we are interested in critical aspect of such movements: contact of a hand
with a target. The questions we attempt to address are:

1. Are primate SC neurons activated for a goal-directed contact?
2. If yes, is the activation simply somatosensory or relates specifically to the self-
induced contact with the target?
3. Does the contact force influence the putative activity of SC neurons?
4. How do SC neurons behave in the presence of external perturbation that causes
sensation that cannot be predicted on the basis of the motor program?

We will address these questions by employing extracellular single neuron
recordings in the primate SC during a goal-directed arm movement experimental
paradigm. The paradigm will involve a trained rhesus monkey subject pressing
illuminated target on the working panel. In addition, an originally designed real time
force perturbation system capable of moving the working panel upon the contact will
be used and the perturbation will be introduced unexpectedly on some trials. Finally,
the contact force and the panel position will be measured and these signals used to
examine the nature of the SC neuronal activity.
RESULTS

Activity of single neurons was extracellularly recorded from the superior colliculus of the rhesus macaque subject during an arm-movement task (see Methods). To initiate a task trial the subject put a hand on the start position in a horizontal plane and fixated a dot of light that appeared shortly thereafter on a vertical working panel. One button on the panel was then lit red followed by a change to green, which was a ‘go’ signal to press the button. After pressing and maintaining the contact, the subject finished the trial by returning the hand to the start position. During the trial visual fixation was required. The liquid reward was delivered upon returning the hand to the start button and maintaining visual fixation for an additional brief period of time. In the extended version of the task, perturbation was introduced during the contact with the target button: on 50% of trials, an abrupt, brief motion of the working panel was unexpectedly triggered upon the contact. The subject was required to maintain normal performance including visual fixation.

During the task 134 neurons in the SC were recorded. These include neurons with activity enhanced (14), or suppressed (1) during the arm movement phase; enhanced (18), or suppressed (2) during the contact phase; modulated by perturbation (30), with a subpopulation activated almost exclusively for perturbation (10). The rest of neurons showed no robust modulation in the task that we could specifically classify (such as neurons activated only at the beginning and end of the trial or suppressed during the performance in the trial, while having high baseline activity otherwise). In addition to these, we observed different neurons that were activated out of the task, mostly when the subject decided not to perform in the task. Those include single cases of neurons reliably firing for placing a hand close to the subject’s face and abruptly stopping upon the hand withdrawal, entering the space
immediately around the subject (without visual responses otherwise), walking in the
visual field of the subject or suppressed by abrupt sounds such as hand clapping.

Here we present two classes of neurons: one activated for a hand-target
contact and the other, for task perturbation. The first group we denominate contact
neurons, and the second perturbation neurons.

Contact Neurons

In the deep lateral zone of the Superior Colliculus we found neurons activated
for the contact with the target within an arm-movement task (Figure 2).

![Figure 2](image)

**Figure 2. Neuronal activity for a goal-directed contact.**

Every dot represents one spike of the example neuron, and every row of
dots corresponds to one trial. Every triangle represents a time point when
a specific event occurred in a trial (green: the arm movement onset, red:
the target contact, green: the onset of the arm movement back to, and
orange: the contact with the start bar at the end of the trial).

A neuron is recognized as a ‘contact neuron’ if it exhibited a vigorous spike
burst for the contact with the target and sparse or no spikes otherwise, which applies
to eight neurons. These neurons typically did not respond to task-irrelevant
somatosensory stimulation of the hand (such as touching monkey’s hand).
Location of Contact Neurons

Contact neurons (100%, 8 out of 8) were found in the deep, lateral part of the SC (Figure 2, Table 1). To minimize invasiveness of the recording we did not penetrate the brain with the guide tube but the electrode was driven the whole path from the dura to the midbrain (see Methods). Upon entering the SC, the location of the visual receptive field was mapped and for all contact neurons receptive fields were located below the horizon indicating that the penetrations were in the lateral SC (Figure 2, Table 1). In addition, contact neurons were located below 2 mm relative to the SC surface, indicating that the recordings were in the deep SC layers (Table 1).

Activation for a Goal-Directed Contact

Contact neurons appeared activated only for a goal-directed contact with the explicit target of the arm movement. Namely, their neuronal activity was weak or absent for a contact with the start button at the end of a trial (e.g. Figure 2) despite this event presents strong somatosensory stimulation. To quantify neuronal activity for the target contact and compare it with the one occurring for the other contact, spikes were counted in the time window starting from the arm movement onset (arm movement towards the target) until the contact offset (onset of the arm movement back to the start button). Namely, contact neurons started to be active at the time of the contact or immediately before (see the next section) and were consistently not active at and around the time of the arm movement onset (neither before nor upon the arm movement, e.g. Figure 2). Thus, the described time window captured the spike burst occurring temporally tied to the contact with the target. Similarly, spikes occurred around the start bar contact were counted in the time window starting from the onset of the arm movement back to the start bar until the end of the trial; this time window captured neuronal activity temporally tied to the start bar contact since the
contact neurons were not active during the arm movement but only at the time of the start bar contact or immediately before.

Figure 3. Receptive Field Coordinates of Visual Neuronal Responses from the Superficial Layers of the SC for Penetrations That Subsequently Acquired Contact Neurons in the Deep SC Zone.

For every penetration into the SC, the receptive field (RF) of neuronal responses to the visual stimulus (see Methods) was determined immediately upon entering the SC. The circular diagram presents a central visual field in front of the subject; the center of the diagram corresponds to the point of the visual fixation. Numbers on the solid circle correspond to the angle in the vertical plane of the field, and numbers on the dashed circles to the visual angle (the angle between the visual stimulus and the center of the visual field). The centers of the neuronal RFs are presented as the red dots.
The neuronal activity for the start bar, if present at all, was manifold weaker than the one for the target contact (Figure 4 and 5).

### Depth Below the SC

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Surface</th>
<th>Visual Angle; Angle to the Horizon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y20015</td>
<td>3,6</td>
<td>17; -50</td>
</tr>
<tr>
<td>Y20037</td>
<td>2,8</td>
<td>15; -60</td>
</tr>
<tr>
<td>Y30051</td>
<td>2,91</td>
<td>7; -55</td>
</tr>
<tr>
<td>FTS012</td>
<td>3,1</td>
<td>21.8; -80</td>
</tr>
<tr>
<td>FTS023</td>
<td>2,65</td>
<td>13; -75</td>
</tr>
<tr>
<td>FTS076</td>
<td>2,61</td>
<td>17.8; -90</td>
</tr>
<tr>
<td>FTS159</td>
<td>2,77</td>
<td>9; -60</td>
</tr>
<tr>
<td>FTS175</td>
<td>4,11</td>
<td>20; -45</td>
</tr>
</tbody>
</table>

**Table 1.** *Contact Neurons Location within the Superior Colliculus.* The first column contains laboratory labels of the recorded neurons, the second depths of the neurons relative to the SC surface, and the third coordinates of the receptive fields for neuronal responses to the visual stimulus immediately upon entering the SC for the penetration in which the neuron was acquired (see also Figure 3). For all neurons the coordinates (visual angle, angle to the horizon) were reliably measured except for FTS175 that were estimated from the drawing in the laboratory protocol.

**Neuronal Latency of Contact neurons**

A specific insight into the nature of the contact neurons’ activity is provided by their neuronal latency (Figure 6). The contact was detected by a sensitive force detector able to detect an impact on the working panel as a whole and not only on the target button (see Methods). This is important because the contact may first take place in the region surrounding the button, slightly before the button is actually
Figure 4. Neuronal Activity for a Contact with a Target vs. with a No-Target: Individual Neurons

Each panel corresponds to one contact neuron. The left bars present the number of spikes which occurred around the time of the target contact. The right bars present the number of spikes which occurred around the time of the start bar contact. Errorbars present standard error of the mean (SEM). Laboratory labels of individual neurons are provided on the horizontal axis.
pressed. In this way, accurate information about the time of the contact was obtained (Figure 6). In addition, we tested the response time of the sensor by comparing the delay of its output signal to the hand arrival time.

![Bar graph showing normalized number of spikes for Target Contact and Start Bar Contact.](image)

**Figure 5.** *Neuronal Population Activity for a Contact with a Target vs. with a No-Target: Population Response*

The left bar represents normalized number of spikes occurred around the time of the target contact (after the arm-movement onset towards the target until the contact offset). The right bar represents normalized number of spikes occurred around the time of the start bar contact (after the arm-movement onset while returning the hand back to the start bar until the end of the trial). The numbers of spikes are normalized by dividing with the number of spikes for a target contact. Errorbars represent standard error of the mean (SEM); n=8 neurons.

Namely, we positioned a laser beam to pass immediately in front of the target button parallel to the working panel and project onto the photocell (see Methods). The hand arriving on the target button interrupted a laser beam and the corresponding photocell signal served as a time reference point. The force sensor output signal was delayed less than 5 ms relative to this reference. Since, the
photocell signal onset occurred before the contact (at the time of the hand arrival) the force sensor delay is certainly below the measured 5 ms. Therefore, possible delays in the force measurement do not underlie the observed phenomenon of advanced neuronal activity.

![Graph showing neuronal activity and force profile](image)

**Figure 6. Neuronal Activity Starting Immediately Before the Contact.** In the upper panel, every dot represents one action potential of the example neuron, every row of action potentials corresponds to one trial, and every triangle represents a time point when a specific event occurred in a trial (the arm movement onset, the target contact, the onset of the arm movement back to, and the contact with the start bar at the end of the trial). In the lower panel, every line represents a force profile during one trial and every dot one action potential; every row of action potentials corresponds to one trial and these action potentials are from the same trial as the force profile immediately above them.
The spike train onset time was tested for difference from zero (time zero corresponds to the contact time). First, the set of latencies was tested for normality by Lilliefors test and if the hypothesis that the distribution of latencies is normal could not be rejected at the 0.05 significance level, one-sample Student T-test was used; otherwise Wilcoxon signed rank test was used.

![Neuronal Latency upon the Contact](image)

**Figure 7. Neuronal Latency upon the Contact**

Each bar corresponds to one contact neuron. The height of a bar presents median neuronal latency and an errorbar demarcates the interquartile range. Laboratory labels of individual neurons are provided on the horizontal axis. Neuronal latency was computed as the time of the first spike in the spike train minus the time of the contact. This approach was verified by binning spike times in 20 ms bins and finding a first time bin containing significantly more spikes than bins corresponding to the baseline neuronal activity. A negative latency indicates that the neuron started to be active in advance of the contact.

Two of seven neurons started to be active significantly after the contact ($p<0.01$, One-Sample Student T-Test). Importantly, spike trains of five out of seven contact neurons start without a delay upon the contact (Table 2).
<table>
<thead>
<tr>
<th>Laboratory Label of a Neuron</th>
<th>Neuronal Latency upon the Contact [ms]</th>
<th>Is the latency different from zero?</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y20015</td>
<td>median: -11.9250  min: -63.5400  max: 70.6200</td>
<td>NO.</td>
<td>0.6644</td>
</tr>
<tr>
<td>Y20037</td>
<td>median: -62.7300  min: -98.9500  max: 25.5300</td>
<td>YES.</td>
<td>1.8891·10^{-6}</td>
</tr>
<tr>
<td>Y30051</td>
<td>median: 28.3250   min: -18.9900  max: 61.4000</td>
<td>YES.</td>
<td>9.6314·10^{-5}</td>
</tr>
<tr>
<td>FTS012</td>
<td>median: 148       min: 76        max: 483</td>
<td>YES.</td>
<td>0.0013</td>
</tr>
<tr>
<td>FTS023</td>
<td>median: -25.5000  min: -96       max: 68</td>
<td>NO.</td>
<td>0.2702</td>
</tr>
<tr>
<td>FTS076</td>
<td>median: -19       min: -85       max: 105</td>
<td>YES.</td>
<td>0.0471</td>
</tr>
<tr>
<td>FTS159</td>
<td>median: 109       min: 23        max: 248</td>
<td>YES.</td>
<td>1.1716·10^{-9}</td>
</tr>
<tr>
<td>FTS175</td>
<td>median: -25       min: -74       max: 14</td>
<td>YES.</td>
<td>0.0097</td>
</tr>
</tbody>
</table>

**Table 2. Neuronal Latencies upon the Contact**

The first column provides laboratory labels of the recorded neurons; the second, third and fourth median, minimal, and maximal neuronal latency upon the contact, respectively. The last column corresponds to the significance test outcome whereby the spike train onset time was tested for difference from zero (time zero corresponds to the contact time); for neuron FTS012 Wilcoxon Signed Rank Test was applied (since the hypothesis about data normality was rejected by Lilliefors test at the 0.05 significance level) and for all others One-Sample Student T-Test.
Three of those start to be active significantly before the contact ($p<0.01$, One-Sample Student T-Test). Therefore, the activity of contact neurons does not appear to be simply a somatosensory response.

**Influence of Perturbation on Contact neurons**

We established that contact neurons are active for the goal-directed contact with the target and that some of them start to be active immediately before the contact. Now we examine how these neurons behave in the presence of the external event, whereby the sensation is not solely a result of the own movement but has an additional external component. On fifty percent of trials, a task perturbation was introduced whereby the working panel abruptly moved upon a subject’s contact with the panel (see Methods). The motion was brief and unexpected for the subject.

In four contact neurons perturbation induced enhancement of neuronal activity (Figure 8), in one suppression (Figure 9) and in two neurons induced virtually no effect. Overall, in five out of seven neurons, perturbation significantly modulated neuronal activity (Table 3, Figure 10).

**Relationship between Force Applied on the Target and Neuronal Activity of Perturbation Neurons**

To examine the relationship between the neuronal activity and the force applied on the target, the two were examined for correlation on a trial by trial basis. Pearson linear correlation was performed and its significance level was tested using a Student’s T distribution for a transformation of the correlation (see Methods). Before deciding which type of the correlation to use, the data set was tested for normality (Lilliefors test); since the normality could not be rejected at the 0.05 significance level, the Pearson linear correlation was used. Force measurements were available for six neurons (see Methods), which are included in the following
Figure 8. **Influence of Perturbation on Contact Neuron**

Every dot represents one action potential of the example neuron, and every row of dots corresponds to one trial. Every triangle represents a time point when a specific event occurred in a trial [from left to right: the arm movement onset (green), the target contact (red), the onset of the arm movement back to (green), and the contact with the start bar at the end of the trial (orange)]. Upper and lower panels correspond to standard and perturbed trials, respectively.
Figure 9. **Influence of Perturbation on Contact Neuron: Suppression**

Every dot represents one spike of the example neuron, and every row of dots corresponds to one trial. Every triangle represents a time point when a specific event occurred in a trial [from left to right: the arm movement onset (green), the target contact (red), the onset of the arm movement back to (green), and the contact with the start bar at the end of the trial (orange)]. Upper and lower panels correspond to standard and perturbed trials, respectively.

analysis. To assure a correct cross-correlation procedure, the neuronal activity and the force were aligned in time. Specifically, for each trial both time arrays were cut to the same length before the contact as well after the contact (see Methods). In this
<table>
<thead>
<tr>
<th>Neuron</th>
<th>Effect of Perturbation</th>
<th>Statistical Significance of the Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y20015</td>
<td>Suppression</td>
<td>$p=1.1378 \times 10^{-7}$, Two-Sample T-Test</td>
</tr>
<tr>
<td>Y20037</td>
<td>No effect</td>
<td>$p=0.2830$, Two-Sample T-Test</td>
</tr>
<tr>
<td>Y30051</td>
<td>Enhancement</td>
<td>$p=1.1978 \times 10^{-5}$, Two-Sample T-Test</td>
</tr>
<tr>
<td>FTS012</td>
<td>Enhancement</td>
<td>$p=2.3710 \times 10^{-10}$, Wilcoxon rank sum test</td>
</tr>
<tr>
<td>FTS023</td>
<td>Enhancement</td>
<td>$p=1.7798 \times 10^{-8}$, Two-Sample T-Test</td>
</tr>
<tr>
<td>FTS076</td>
<td>No effect</td>
<td>$p=0.0872$, Two-Sample T-Test</td>
</tr>
<tr>
<td>FTS159</td>
<td>Enhancement</td>
<td>$p=1.5385 \times 10^{-4}$, Two-Sample T-Test</td>
</tr>
<tr>
<td>FTS175</td>
<td>No effect</td>
<td>$p=0.5245$, Two-Sample T-Test</td>
</tr>
</tbody>
</table>

**Table 3.** *Influence of Perturbation on Contact Neurons: Data Summary*

The first, second, and third column provide laboratory labels of the recorded neurons, effect description (difference in number of spikes on perturbed vs. unperturbed trials), and its statistical significance level together with the applied test, respectively.
Figure 10.  
**Influence of Perturbation on Contact Neurons**

Each panel corresponds to one contact neuron having neuron's laboratory label provided on the top. The left and right bars within each panel represent mean number of spikes occurred in perturbed and unperturbed trials, respectively. Errorbars represent standard error of the mean (SEM).
way, all time arrays were made equally long and aligned to the contact time as a reference time point.

First, the correlation was examined between number of spikes which occurred for the contact and the peak force on individual trials. Spikes were counted in the time window starting from the arm movement onset (arm movement towards the target) until the contact offset (onset of the arm movement back to the start button). Namely, contact neurons started to be active at the time of the contact or immediately before (the earliest start of the spike train across all neurons and all trials was -98.95 ms relative to the contact) and were consistently not active at and around the time of the arm movement onset (neither before nor upon the arm movement, e.g. Figure 2). Thus, the described time window captured the spike burst occurring temporally tied to the contact with the target. The peak force always occurred immediately following the contact, corresponding to the initial impact due to the abrupt deceleration of the hand upon the contact. The number of spikes was significantly correlated with the peak contact force for two neurons (Table 4).

Second, a correlation was examined between the peak instantaneous spiking frequency and the peak contact force on individual trials. The instantaneous spiking frequency of an individual trial was first smoothed by running average algorithm (see Methods). The smoothing preserved all features of the spike frequency function and only removed extreme fluctuations. The peak of the smoothed instantaneous spiking frequency was significantly correlated with the peak force for one neuron (Table 4). The same neuron had both correlations (number of spikes vs. peak contact force and peak instantaneous spiking frequency vs. peak contact force) significant ($p<0.05$).

Third, a smoothed instantaneous spiking frequency was cross-correlated with the force on a trial by trial basis. First, for each unperturbed trial, one cross-correlogram was computed and then a mean value calculated for all these trials.
### Table 4. Contact Neurons: Correlation of Neuronal Activity and Force

The first column provides laboratory labels of the recorded neurons, the second correlation coefficients for correlation between number of spikes occurred around the contact time vs. the peak force occurred during the contact (the peak force always occurred immediately following the contact, corresponding to the initial impact due to the abrupt deceleration upon the contact), the third \( p \) values (a statistical significance level) for preceding correlation coefficients, the fourth correlation coefficients for peak instantaneous spiking frequency vs. the peak force occurred during the contact, and the last \( p \) values (significance levels) for preceding correlation coefficients. Correlations having \( p \) values below 0.05 are in bold print. Only unperturbed trials are included.
(Figure 11). Obtained correlation was moderate across all neurons (Figure 12). The same analysis was performed for perturbed trials (Figure 13) revealing moderate correlation across neurons (Figure 14). Correlation between neuronal activity and contact force was higher in perturbed than in unperturbed trials (Figure 15): Two-way ANOVA revealed significant effect of the trial type (unperturbed vs. perturbed) on the correlation \( (p=2.4660\cdot10^{-10}) \). Although correlation tended to be different across neurons, this trend was not significant: there was no significant effect of the neuron on the correlation \( (p=0.0593) \). There was a significant interaction between the neuron identity and the trial type \( (p=0.0023) \). Importantly, in perturbed trials one part of the force is caused by perturbation thus having an external cause (not generated by animal’s own movement) – this external force component may correlate with the neuronal activity contributing towards the tendency for higher correlation in perturbed trials.

Finally, we performed cross-correlation on super arrays formed by concatenating individual trials. First, for each neuron smoothed instantaneous frequency from all unperturbed trials was concatenated in one super array (see Methods) as well as force time profiles from unperturbed trials, and the two obtained super arrays were cross-correlated (Figure 16). This revealed moderate to high correlation on unperturbed trials across neurons (Figure 17). The same procedure was performed for perturbed trials (Figure 18) also revealing moderate to high correlation across neurons (Figure 19). Also in this case correlation coefficients were significantly higher for perturbed than for unperturbed trials (Figure 20; \( p=0.0062 \), Two-sample T test, Figure 21).
Figure 11. Cross-Correlation on a Trial by Trial Basis: Neuronal Activity vs. Contact Force, Unperturbed Trials

Each gray line represents a single trial cross-correlogram of the neuronal activity and the force for the example neuron (FTS076). The bold black line represents the mean of all the trials. All correlations are significant with \( p < 10^{-8} \).

Figure 12. Cross-Correlation of Neuronal Activity vs. Contact Force on a Trial by Trial Basis: Correlation Coefficients for Unperturbed Trials

Each bar corresponds to one contact neuron, whose laboratory label is provided below the bar. A height of a bar presents a mean correlation coefficient for unperturbed trials (derived from cross-correlograms of individual unperturbed trials). Errorbars present standard error of the mean. All correlations are significant with \( p < 10^{-8} \).
Figure 13. *Cross-Correlation on a Trial by Trial Basis: Neuronal Activity vs. Contact Force on Perturbed Trials*

Each gray line represents a single trial cross-correlogram of the neuronal activity and the force for the example neuron (FTS076). The bold black line represents the mean of all the trials. All correlations are significant with $p<10^{-8}$.

Figure 14. *Cross-Correlation of Neuronal Activity vs. Contact Force on a Trial by Trial Basis: Correlation Coefficients for Perturbed Trials*

Each bar corresponds to one contact neuron, whose laboratory label is provided below the bar. A height of a bar presents a mean correlation coefficient for perturbed trials (derived from cross-correlograms of individual perturbed trials). Errorbars present standard error of the mean. All correlations are significant with $p<10^{-8}$.
Figure 15. *Mean Correlation Coefficients of Neuronal Activity and Contact Force on a Trial by Trial Basis: Unperturbed vs. Perturbed Trials.*

Each dot corresponds to one neuron and represents the pair of mean correlation coefficients for unperturbed (horizontal axis) vs. perturbed (vertical axis) trials. The dashed line represents the line of unity.

To test whether local fluctuations within neuronal activity covary with local fluctuations in force, a ‘shuffle predictor’ based algorithm was used (see Methods). Namely, a new super array with smoothed instantaneous spiking frequency was formed having the order of individual trials changed. Then, this shuffled super array was cross-correlated with the unchanged super array for force. First, the analysis was performed for unperturbed trials (*Figure 20*, compare with *Figure 21*).
Figure 16. Cross-Correlation of Neuronal Activity vs. Contact Force: Super Arrays of Single Unperturbed Trials

The uppermost panel represents instantaneous spiking frequency of the example neuron (FTS076) on unperturbed trials concatenated in one temporal array. The middle panel represents contact force occurred during the same trials, concatenated in one temporal array. The lowest panel represents cross-correlogram of the two arrays.

Figure 17. Cross-Correlation of Neuronal Activity vs. Contact Force: Super Arrays of Single Unperturbed Trials

Each bar corresponds to one contact neuron, whose laboratory label is provided below the bar. A height of a bar represents a correlation coefficient derived from the cross-correlogram of spiking and force super arrays (see Figure 16). Errorbars represent standard error of the mean. All correlations are significant with \( p < 10^{-8} \).
Figure 18. Cross-Correlation of Neuronal Activity vs. Contact Force: Super Arrays of Single Perturbed Trials

The uppermost panel represents spiking frequency of the example neuron (FTS076) on perturbed trials concatenated in one temporal array. The middle panel represents contact force occurred during the same trials, concatenated in one temporal array. The lowest panel represents the cross-correlogram of the two arrays.

Figure 19. Cross-Correlation of Neuronal Activity vs. Contact Force: Super Arrays of Single Perturbed Trials

Each bar corresponds to one contact neuron, whose laboratory label is provided below the bar. A height of a bar presents a correlation coefficient derived from cross-correlograms of spiking and force super arrays on perturbed trials (see Figure 21). All correlations are significant with $p<10^{-8}$. 
**Figure 20.** Correlation Coefficients of Neuronal Activity and Contact Force on Super Arrays: Unperturbed vs. Perturbed Trials

Each dot corresponds to one neuron and represents the pair of correlation coefficients for unperturbed (horizontal axis) vs. perturbed (vertical axis) trials obtained from the cross-correlation of instantaneous spiking frequency and force super arrays. The dashed line represents the line of unity.

**Figure 21.** Cross-Correlation of Neuronal Activity vs. Contact Force Super Arrays on Unperturbed and Perturbed Trials across All Neurons

The left and right boxplots correspond to the correlation coefficients at the optimal lag obtained from the cross-correlograms of neuronal activity vs. contact force super arrays (see **Figures 16** and **18**), across all neurons for unperturbed and perturbed trials, respectively. The red horizontal lines represent median, the blue boxplots demarcate the interquartile range, and the whiskers embrace all data points. The two correlation coefficients are significantly different ($p=0.0062$, Two-sample T test).
Across the neurons correlation stayed moderate to high (Figure 22, compare with Figure 20). Then, the analysis was performed for perturbed trials (Figure 23, compare with Figure 21). Across the neurons correlation stayed moderate to high (Figure 24, compare with Figure 22). Correlation coefficients for shuffled super arrays on perturbed trials were significantly different than on unperturbed trials (p=0.0011, Two-sample T test). Importantly, if covariance of local fluctuations in neuronal activity and the force underlies high correlation between the two then the cross-correlation of the super arrays with mismatched trials should yield significantly lower correlation. However, correlation coefficients on shuffled super arrays were not significantly different from those on original super arrays for neither unperturbed (p=0.5994, Two-sample T test, Figure 25) nor perturbed trials (p=0.6835, Two-sample T test, Figure 26). Thus, the obtained relatively high correlation of the two arrays does not reflect their local covariance but rather appear to reflect global congruence of their non-zero epochs in time.
Figure 22. Cross-Correlation of Neuronal Activity vs. Contact Force: Shuffled Super Arrays of Unperturbed Trials

The uppermost panel represents spiking frequency of the example neuron (FTS076) on unperturbed trials. The trials are shuffled and concatenated in one temporal array. The middle panel represents contact force from the same trials, concatenated in one temporal array in the genuine order. Importantly, because trials in the spiking frequency array are shuffled, trials from the two arrays do not match any more (difference with Figure 16). The lowest panel represents cross-correlogram of the two arrays.

Figure 23. Cross-Correlation of Neuronal Activity vs. Contact Force: Shuffled Super Arrays of Unperturbed Trials

Each bar corresponds to one contact neuron (the laboratory label below the bar). A height of a bar presents a correlation coefficient ($p<0.001$) derived from cross-correlogram of shuffled spiking super array and veridical force super array on unperturbed trials (see Figure 22).
Figure 24. Cross-Correlation of Neuronal Activity vs. Contact Force: Shuffled Super Arrays of Perturbed Trials

The uppermost panel represents spiking frequency of the example neuron (FTS076) on perturbed trials. The trials are shuffled and concatenated in one temporal array. The middle panel represents contact force from the same trials, concatenated in one temporal array in the genuine order. Importantly, because trials in the spiking frequency array are shuffled, trials from the two arrays do not match any more (difference with Figure 18). The lowest panel represents cross-correlogram of the two arrays.

Figure 25. Cross-Correlation of Neuronal Activity vs. Contact Force: Shuffled Super Arrays of Perturbed Trials

Each bar corresponds to one contact neuron (the laboratory label below the bar). A height of a bar presents a correlation coefficient ($p<0.001$) derived from cross-correlogram of shuffled spiking super array and veridical force super array on perturbed trials (see Figure 24).
Figure 26. Cross-Correlation of Neuronal Activity vs. Contact Force: ‘Shuffled Predictor’ Analysis for Unperturbed Trials across All Neurons

The left boxplot corresponds to the correlation coefficients at the optimal lag obtained from the cross-correlogram of the two super arrays: one comprising spiking frequency on individual unperturbed trials concatenated, and the other contact force on individual unperturbed trials concatenated. The right boxplot corresponds to the correlation coefficients at the optimal lag obtained from the cross-correlogram of the two super arrays: one comprising spiking frequency on individual unperturbed trials shuffled and then concatenated and the other contact force on individual unperturbed trials concatenated in a genuine order (see Figure 24). The red horizontal lines represent median, the blue boxplots demarcate the interquartile range, and the whiskers embrace all data points. The two correlation coefficients are not significantly different ($p=0.5994$, Two-sample T test).
Figure 27. Cross-Correlation of Neuronal Activity vs. Contact Force: ‘Shuffled Predictor’ Analysis for Perturbed Trials across All Neurons

The left boxplot corresponds to the correlation coefficients at the optimal lag obtained from the cross-correlogram of the two super arrays: one comprising spiking frequency on individual perturbed trials concatenated, and the other contact force on individual perturbed trials concatenated.

The right boxplot corresponds to the correlation coefficients at the optimal lag obtained from the cross-correlogram of the two super arrays: one comprising spiking frequency on individual perturbed trials shuffled and then concatenated and the other contact force on individual perturbed trials concatenated in a genuine order (see Figure 24). The red horizontal lines represent median, the blue boxplots demarcate the interquartile range, and the whiskers embrace all data points. The two correlation coefficients are not significantly different ($p=0.6835$, Two-sample T test).
**Perturbation Neurons**

To expose the subject to sensation that is not solely a result of his own motor program, a task perturbation was introduced unexpectedly on some trials. On fifty percent of trials, the working panel abruptly moved upon a subject’s contact with the panel (see Methods). The motion was brief and unexpected for the subject. Task perturbation, revealed a novel class of neurons vigorously activated almost exclusively upon perturbation and some of those neurons were undetectable during the standard task (Figure 28). Out of 134 neurons recorded in the task, 30 were modulated by perturbation, and 10 were activated vigorously almost exclusively for perturbation having a spike burst upon the perturbation and sparse or no spikes otherwise. The last group is denominated as perturbation neurons (Figures 28 and 29).

**Location of Perturbation neurons**
Perturbation neurons were found in the lateral zone of the deep SC (Table 5, Figure 30). In the later stage of the study we tried to adjust electrode penetration in order to attain this target zone. The perturbation neurons in the right SC (9 out of 9 recorded in the right SC) were found below 2 mm from the SC surface in the lateral SC zone (Table 5). One perturbation-only neuron in the left SC (1 out of 1 recorded in the left SC) was found also below 2 mm from the SC surface, however the visual receptive field of superficial zone was 80° above the horizon, indicating that the penetration was in the medial SC (Table 5). Notably, minority of penetrations were performed in the left brain hemisphere (8%; 28 out of 341) and those that were confirmed to be in the SC (53%; 15 out of 28) all had visual receptive fields in the upper visual hemifield indicating that the penetrations were in the medial SC.
Figure 28.  Neuronal Activity Emerging Upon Perturbation.  
The uppermost and middle panels show activation of the example neuron during standard and perturbed trials, respectively. Each dot represents a time point when a spike occurred, and each row of dots corresponds to one trial. Each triangle represents a time point when a specific event occurred in a trial (standard trials: the arm movement onset, the target contact, the onset of the arm movement back to, and the contact with the start bar at the end of the trial; perturbed trials: events are the same as for standard trials with the addition of perturbation, which follows the target contact).

The lowest panel shows a typical panel excursion profile (the solid line; displacement is measured at the top of the working panel, which presents the maximal distance from the axis of rotation) together with time points when spikes (the row of dots) occurred in the same trial, aligned in time (the example trial number 4).
Figure 29. Average Neuronal Activation for Population of Neurons on Standard and Perturbed Trials

The left and right columns represent mean number of spikes occurred in standard and perturbed trials, respectively. Spikes were counted from the time point of the contact until the end of the trial. The error bars represent standard error of the mean. The two means are significantly different ($p<0.001$, Wilcoxon rank sum test).

Effect of a Previous Trial

Perturbed trials were analyzed for the influence of a previous trial on the neuronal activation. Specifically, two data groups of perturbed trials were formed: a neuronal activation in a perturbed trial was allotted to one group if the previous trial was perturbed and to another group if the previous trial was unperturbed. If neuronal responses had tendency for a habituation-like effect then the neuronal activation in a perturbed trial preceded by the perturbed trial would tend to be lower as when preceded by an unperturbed trial.
<table>
<thead>
<tr>
<th>Neuron</th>
<th>Depth Below the SC</th>
<th>Visual Angle; Angle to the Horizon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y20023</td>
<td>3,7</td>
<td>17; -45</td>
</tr>
<tr>
<td>Y30011</td>
<td>2,59</td>
<td>15; -70</td>
</tr>
<tr>
<td>Y30036</td>
<td>2,97</td>
<td>8; -50</td>
</tr>
<tr>
<td>Y30069</td>
<td>2,25</td>
<td>15; -55</td>
</tr>
<tr>
<td>FTS031</td>
<td>2,72</td>
<td>8; -90</td>
</tr>
<tr>
<td>FTS138</td>
<td>3,51</td>
<td>17; -70</td>
</tr>
<tr>
<td>FTS187</td>
<td>2,52</td>
<td>10; -90</td>
</tr>
<tr>
<td>FTS191</td>
<td>2,9</td>
<td>10; -75</td>
</tr>
<tr>
<td>FTS206</td>
<td>2,05</td>
<td>16; -80</td>
</tr>
<tr>
<td>FTS221</td>
<td>3,25</td>
<td>17; 80</td>
</tr>
</tbody>
</table>

**Table 5.** Perturbation neurons: Location within the Superior Colliculus.

The first column contains laboratory labels of the recorded neurons, the second depths of the neurons relative to the SC surface, and the third coordinates of the receptive fields for neuronal responses to the visual stimulus immediately upon entering the SC for the penetration in which the neuron was acquired (see also Figure 30). All neurons are from the right superior colliculus except FTS221.
Figure 30. Receptive Field Coordinates of Visual Neuronal Responses from the Superficial Zone of the SC for Penetrations That Subsequently Acquired Perturbation neurons in the Deep SC Zone.

For every penetration into the SC, the receptive field (RF) of neuronal responses to the visual stimulus (see Methods) was determined immediately upon entering the SC. The circular diagram presents a central visual field in front of the subject; numbers on the outer circle correspond to the angle in the field, and concentric circles correspond to the visual angle (the angle between the visual stimulus and the center of the visual field) in steps of 10 degrees. The centers of the neuronal RFs are presented as the red dots.
However, this was not the case (Figure 31). The mean number of spikes in a perturbed trial preceded by an unperturbed trial was not significantly different from the mean number of spikes on a perturbed trial preceded by a perturbed trial ($p=0.5751$, Two-sample T test). In addition, we examined number of action potentials in pairs of two subsequent perturbed trials (Figure 32). If the neuronal response has a tendency for a habituation-like effect, the subsequent perturbed trial would tend to contain lower number of spikes. However, there was no significant difference of the subsequent perturbed trials pairs’ distance from the unity line ($p=0.4902$, Wilcoxon signed rank test).

![Figure 31](image-url)

**Figure 31. Effect of a Previous Trial on Neuronal Activity in a Perturbed Trial.**

The left and the right bar represent mean number of action potentials occurred in a perturbed trial if a previous trial was perturbed and if a previous trial was unperturbed, respectively. There is no significant difference between the two groups ($p=0.5751$, Two-sample T test). Action potentials were counted starting from the contact time until the end of the trial. The errorbars represent the standard error of the mean (SEM).
This analysis could be potentially performed on more subsequent perturbed trials; however, during the task perturbed trials occur pseudo-randomly (see Methods) and an individual perturbed trial can be followed by a missed (e.g. because the subject broke visual fixation), an unperturbed or a perturbed trial. Due to the limited time available to collect data from one neuron, arrays of subsequent perturbed trials for an individual neuron occurred seldom and potential trends for arrays of more than two subsequent perturbed trials are not statistically significant.

Figure 32. *Effect of the Perturbed Trial on the Neuronal Activity in the Next Perturbed Trial.*

Each dot represents one pair of subsequent perturbed trials, the dashed line the line of unity, the center of the red cross the mean of all pairs and its horizontal and vertical lines correspond to the standard error of the mean (SEM) for trials N and N+1, respectively. Included are data from ten neurons.
Neuronal Latency of Perturbation Neurons

One critical property that characterise these neurons and distinct them from other classes of neurons reported for the SC is a long neuronal latency (Figure 33). The onset of perturbation is here defined as the time point when the sound produced by the system that moves the working panel, which is a first signal of perturbation, can be detected by the subject. Namely, a perturbed trial comprises a following time course of events: the computer sends a trigger for perturbation upon the subject pressed the target button on the working panel; 15 ms afterwards the automatic valves open, which produces a sound; the sound travels 8 ms to reach the subject’s ears; approximately 125 ms later (depending on the push force) the panel starts to move. In order to determine the neuronal latency upon perturbation we computed the onset of neuronal activity upon the onset of perturbation using three algorithms and verified their compliance (see Methods). First, we calculated a time period between the onset of perturbation and the time point when the SIF reached half of the peak value. Second, we counted number of spikes within 20 ms time bins and deemed a time of a first bin containing a number of spikes higher by 3 standard deviations than the median number of spikes before the perturbation as a neuronal activity onset, consequently providing neuronal latency. Finally, we acknowledged the time difference between the first spike produced upon the perturbation onset and the perturbation onset as a neuronal latency. The two later approaches gave similar results that did not differ significantly across neurons ($p=0.9816$, Two-Sample T test; binning - 1st spike: median -3.75 ms, interquartile range from -9.65 ms to 2 ms, min -21.5 ms, max 11 ms with the exception of one outlier at 24.52 ms). In addition, they were in compliance with estimates obtained by visual inspection and we adopted them as plausible. First approach gave too long latencies and was not further considered. In addition, the neurons typically had zero baseline activity with
occasional individual spikes or no spikes at all in the absence of perturbation. This resulted, amongst other, in an excellent compliance of the last two approaches.

Figure 33. Neuronal Latency upon the Perturbation Sound
Each bar corresponds to one perturbation-only neuron. Height of a bar presents median neuronal latency and an errorbar demarcates an interquartile range. Neuronal latency for each neuron was computed by forming a 20 ms time bins within an each trial and finding a first bin upon perturbation that contained a number of spikes exceeding the number of spikes before the perturbation by three standard deviations. In addition, the time of the first spike relative to the perturbation was determined and such latencies were in compliance with those obtained by binning approach.

Control Tasks: Passive Observation of Perturbation
To examine whether perturbation neurons respond to the audiovisual features of the perturbation alone we introduced two control tasks.

In the first one, the subject was exposed to the event of perturbation without touching the working panel (see Methods). The subject’s arms were loosely
restrained and the motion of the working panel was triggered after a delay following the green ‘go’ signal on 50% of trials unexpectedly as in the original task. During the trial a visual fixation was required and this control task was overall identical to the original one except for the absence of the arm-movement. Four neurons identified as having properties of perturbation neurons in the original task were tested in the control task. This exposure to the perturbation event elicited neuronal responses (Figure 34).

In the second control task, the perturbation was triggered on 50% of the trials unexpectedly, however the working panel was fixed. Hence, there was no motion of the panel, but the subject was exposed to the perturbation sound. The same four neurons tested in the first control task were also tested in this second control task. Such exposure elicited neuronal responses, as well (Figure 35).

If the signal from the neuron was stable sufficiently long we additionally tested perturbation neurons with task-irrelevant sensory stimuli. These included flashing dot stimuli and moving objects such as a board of a similar size as the working panel towards and away from the subject. These stimuli typically did not elicit responses. Furthermore, these tests comprised a variety of auditory stimuli including sound bursts that resembled but were not identical to the sound of perturbation (see Methods). These stimuli as well did not elicit responses. Together, this suggests that these neurons are not simply sensory neurons. The only stimulus that elicited responses out of the task was the motion of the working panel itself. Namely, two neurons that were tested in the control tasks were also tested by having the panel being moved by the researcher. During this, the subject was freely looking around and was not required to pay attention. One of the two neurons exhibited some response and the other did not. In addition, one neuron that was not tested in the control tasks was
Figure 34.  

Control Task 1: Subject Observes the Event of Perturbation

Neuronal activity of an example neuron (FTS206) is shown for the original task (the upper two panels) and for the Control Task 1 (the lower two panels). The original task was performed first, followed by the Control Task 1.
Figure 35. Control Task 2: Subject Audits the Event of Perturbation

Neuronal activity of the example neuron (the same neuron as in the Figure 36) is shown for the original task (the upper two panels) and for the Control Task 2 (the lower two panels). The original task was first performed followed by the Control Task 1, and then Control Task 2.
presented with such panel motion and the observed response was strong. For the
hand motion, its response was weak or absent.

It appears that only stimuli identical to those contained in the perturbation
event may elicit responses. In addition, the control tasks were introduced after
subject acquired extensive experience in the task. Thus, the observed neuronal
activation may have emerged due to association (see Discussion).

Relationship between Contact Force and Neuronal Activity of
Perturbation Neurons

Since perturbation neurons are activated in perturbed trials and not in
unperturbed, we examine how neuronal activity may correlate with external stimuli
that are different between the two trial types. These stimuli can be calculated at the
average level across trials. Specifically, the difference between means of measured
contact force on perturbed vs. unperturbed trials, as well as the difference between
means of the contact force time derivative present such putative stimuli. As a
continuous time function representation of a neuronal activity, a smoothed
instantaneous firing frequency of a neuron (SIF) was used (see Methods). First,
cross-correlation is performed between the difference of mean SIFs on perturbed vs.
standard trials and the difference of mean force time profiles on perturbed vs.
standard trials, as well as between the difference of mean SIFs on perturbed vs.
standard trials and the absolute difference of mean force time profiles on perturbed
vs. standard trials. At the optimal lag between two cross-correlated time arrays the
cross-correlation function is maximal; when referring to correlation coefficients
obtained from cross-correlograms we refer to those at the optimal lag. Each array
involved in cross-correlation contains several thousand values (usually between three
and five thousand) corresponding to the trial length in ms. Namely, a typical task trial
lasts between three and five seconds, having neuronal activity, as well as force and
panel position represented on the scale with 1 ms resolution. Each correlation coefficient at one lag is consequently highly significant ($p<10^{-8}$).

The cross-correlation analysis reveals a low correlation at the optimal lag for neuronal activity and the force difference on perturbed vs. standard trials (0.1952), however a high correlation for the neuronal activity and the absolute force difference (0.8220, Figure 36).

The cross-correlation analysis is performed for eight perturbation neurons that have the force measurements available, which again reveals a low to moderate correlation at the optimal lag across neurons for neuronal activity and the force difference on perturbed vs. standard trials (median 0.3488), however a high correlation for the neuronal activity and the absolute force difference (median 0.7999, Figure 37 and 38). The correlation coefficients for the two cross-correlations across all neurons are significantly different ($p<0.0005$, Two-sample T-test).

Next, we examine the relationship between the neuronal activity and the force change in time. We perform cross-correlation of the neuronal activity and the derivative of the smoothed force difference between perturbed and standard trials (Figure 39, see Methods). This reveals low to moderate correlation at the optimal lag for the population of neurons (Figure 40).

**Relationship between Panel motion and Neuronal Activity of Perturbation neurons**

The motion of the working panel, which occurred during perturbation, was measured (see Methods) and here we analyze its relationship with the activity of the perturbation neurons. This panel motion induced by perturbation is another external stimulus that differs between perturbed and unperturbed trials. In addition, it is present exclusively during perturbed trials.
Figure 36. Cross-correlation of Mean Neuronal Activity vs. Mean Force Difference between Perturbed and Unperturbed Trials and vs. Absolute Value of the Force Difference

The panels (top to down): Smoothed mean instantaneous spiking frequency (SMIF) of an example neuron (Y30011), the difference of the mean forces in perturbed and unperturbed trials, cross-correlogram of the SMIF and the difference of the mean force in perturbed trials and unperturbed trials, cross-correlogram of the SMIF and the absolute difference of the mean force in perturbed trials and unperturbed trials. All mean values correspond to the same ten trials.
Figure 37. Cross-Correlation of Neuronal Activity vs. Force Difference and vs. Absolute Value of the Force Difference: Correlation Coefficients

Each group of two bars corresponds to one perturbation-only neuron, whose laboratory label is provided below the bar. A height of a bar presents correlation coefficient at the optimal lag derived from the corresponding cross-correlogram: light gray bar – mean neuronal activity vs. difference of mean forces on perturbed and unperturbed trials, dark grey bar – mean neuronal activity vs. absolute value of the difference (see Figure 36).
Figure 38. Cross-Correlation of Difference in Mean Neuronal Activity vs. 1) Difference of Mean Force on Perturbed and Unperturbed Trials, and vs. 2) Absolute Difference of Mean Force on Perturbed and Unperturbed Trials: Correlation Coefficients

The left and right boxplot correspond to the correlation coefficients at the optimal lag obtained from crosscorrelograms for the cases 1) and 2), respectively. Red horizontal lines represent medians, vertical spans of the blue boxplots demarcate the interquartile range, and the whiskers embrace all data points. The red crosses represent data points distant from the median more than 1.5 interquartile ranges beyond the last data point within the interquartile range. The two correlations are significantly different ($p=1.0188\cdot10^{-6}$, Two-Sample T test).
Figure 39. Cross-correlation of Difference in Mean Neuronal Activity vs. Derivative of Mean Force Difference between Perturbed and Unperturbed Trials
The panels (top to down): Smoothed mean instantaneous spiking frequency (SMIF) of an example neuron, derivative of the difference between mean force in perturbed and unperturbed trials, cross-correlogram of the two. All mean values correspond to the same ten trials.

Figure 40. Cross-Correlation of Neuronal Activity vs. Derivative of the Force Difference: Correlation Coefficients
Each bar corresponds to one perturbation-only neuron, whose laboratory label is provided below the bar. A height of a bar presents correlation coefficient at the optimal lag derived from the crosscorrelogram of the mean neuronal activity vs. the derivative of the force difference between perturbed and unperturbed trials.
First, the neuronal activity on perturbed trials and the time profiles of the panel position on perturbed trials were cross-correlated on a trial-by-trial basis (Figure 41). This reveals moderate to high correlation across neurons (the distribution of the mean values from individual neurons: median 0.7634, min 0.6315, max 0.8280, Figure 42).

Second, the neuronal activity on perturbed trials and the time derivative of the panel position on perturbed trials were cross-correlated on a trial-by-trial basis (Figure 43). This reveals low to moderate correlation across neurons (the distribution of the mean values from individual neurons: median 0.4980, min 0.1798, max 0.5589, Figure 44).

Two-way ANOVA suggests that correlation is significantly different between the last two conditions (significant effect of the condition, $p<10^{-21}$), between neurons (significant effect of the neuron, $p=3.0302\cdot10^{-7}$), and that there is interaction between the condition and the neuron ($p=4.4185\cdot10^{-11}$).

**Question of the Neuronal Latency Estimation from Crosscorrelograms**

It is worth emphasizing that optimal lag in cross-correlation often does not present a proper estimate of the onset latency between the two cross-correlated quantities. An ideal case when cross-correlation of the two time functions may provide a proper estimate of latency between the two is when the functions are linearly dependent. In this case, time shapes of the two are the same, only the magnitude is multiplied by a constant. Then, the optimal lag (time lag for which the crosscorrelogram is maximal) presents the latency between the two cross-correlated functions. However, if the time shape of the two functions is not similar enough, the optimal lag obtained from cross-correlation may not present a proper estimate of the latency. The neuronal activity and functions here cross-correlated have different time
Figure 41. *Cross-Correlation on a Trial by Trial Basis: Neuronal Activity vs. Panel Position on Perturbed Trials*

Each gray line represents a single perturbed trial cross-correlogram of the neuronal activity and the panel position for the example neuron (Y30011). The bold black line represents the mean of all the trials. All correlations are significant with $p<10^{-8}$.

Figure 42. *Cross-Correlation of Neuronal Activity vs. Panel Position on a Trial by Trial Basis for Perturbed Trials: Correlation Coefficients*

Each bar corresponds to one perturbation-only neuron, whose laboratory label is provided below the bar. A height of a bar presents a mean correlation coefficient for perturbed trials (derived from cross-correlograms of individual perturbed trials). Errorbars present standard error of the mean. All correlations are significant with $p<10^{-8}$.
Figure 43. *Cross-Correlation on a Trial by Trial Basis: Neuronal Activity vs. Time Derivative of Panel Position on Perturbed Trials*

Each gray line represents a single perturbed trial cross-correlogram of the neuronal activity and the time derivative of the panel position for the example neuron (Y30011). The bold black line represents the mean of all the trials. All correlations are significant with $p<10^{-8}$.

Figure 44. *Cross-Correlation of Neuronal Activity vs. Time Derivative of Panel Position on a Trial by Trial Basis for Perturbed Trials: Correlation Coefficients*

Each bar corresponds to one perturbation-only neuron, whose laboratory label is provided below the bar. A height of a bar presents a mean correlation coefficient for perturbed trials (derived from cross-correlograms of individual perturbed trials). Errorbars present standard error of the mean. All correlations are significant with $p<10^{-8}$. 
profiles and the optimal lag of cross-correlation should not be considered as an mate of latency.
DISCUSSION

In this study, a goal-directed arm movement paradigm that included hand-target contact and external perturbation was used and two novel classes of neurons were found within the deep SC layers.

First, we report neurons vigorously active for a goal-directed contact of a hand with a target. They appear to be activated specifically for a goal-directed contact and not otherwise (e.g. during task-irrelevant somatosensory stimulation of the hand). Their maximal firing rate is typically not correlated with the contact force. Activation in majority of them is modulated by externally applied perturbation. Moreover, a subpopulation of these neurons exhibits a particular property of starting to be active immediately before the contact.

Second, we report neurons vigorously active almost exclusively for the perturbation. They otherwise appeared not responsive to task-irrelevant auditory, visual or somatosensory stimuli. Some neurons exhibited unusually long latencies e.g. exceeding 300 ms relative to the sound as the first detectable sign of perturbation and 200 ms relative to the panel motion onset.

Contact Neurons
Activity of contact neurons seems to be specifically related to the goal-directed contact of the hand with the target and not simply a somatosensory response. Task-irrelevant somatosensory stimulation of the subject’s hand (e.g. touching the subject’s hand by the experimenter) does not elicit responses in these neurons. This is documented in the task, whereby the contact with the start bar upon returning the hand back elicits weak or no activation, while the contact with the specific goal of the arm movement i.e. the target button elicits a vigorous neuronal activation (e.g. Figure 2).
The observed phenomenon may present additional evidence about the interdependence of the sensation and movement in the sensorimotor system. The contact with the target presents a critical event within a goal-directed arm movement and accompanied sensation produced by the hand-target contact has a special meaning that discerns it from a passive sensation. Namely, it is a result of a self-generated movement and this sensation must have a neuronal substrate. The seemingly opposite type of processing has been extensively evidenced in the sensorimotor system — attenuation of sensory signals during self generated movements (for a review see Bays & Wolpert 2007, Cullen 2004, Blakemore et al 2000). In humans, the tactile sensitivity appears to be reduced during active movement of limbs and digits (Angel & Malenka 1982, Chapman et al 1987, Milne et al 1988). When tracking a target with an index finger a detection threshold for a cutaneously applied electrical signal is increased and this increase is higher for higher finger velocities, suggesting lower sensitivity during finger motion (Angel & Malenka 1982). An effect that arm motion increases detection threshold was observed also for electrical stimulation of the forearm for both active and passive movements having higher and more consistent effect for active movements (Chapman et al 1987). In the case of artificially delayed execution of the planned movement, the sensory attenuation may be observed the intended time of the movement: Transcranial magnetic stimulation focused onto the primary motor cortex can delay execution of a planned finger movement and upon delivery of such stimulation the attenuation of the cutaneous stimuli occurs well in advance of the eventual movement at the putative intended time of the movement (Voss et al 2006). In monkeys, related effects were evidenced by reduced evoked responses upon electrical cutaneous stimulation in the lemniscal system during active limb movements (Dyhre-Poulsen 1978). In addition, there is evidence suggesting that
somatosensory transmission may be attenuated by top-down signal prior and during the arm movement in the dorsal column nuclei, thus at the first synapse stage in the somatosensory pathway (Chapman et al. 1988). Furthermore, the attenuation appeared to be present at the level of the caudal division of the ventral posterior lateral nucleus of the thalamus, and in the somatosensory cortex, whereby the decrease of the response was the most pronounced in the somatosensory cortex (Chapman et al. 1988). On the other hand, passive movements appeared to result in attenuation at the level of the somatosensory cortex and the thalamus, however not at the level of the medial lemniscus (Chapman et al. 1988). There is evidence from a more recent study that motor commands may attenuate the afferent somatosensory signals at the level of the first synapse in the spinal cord. Specifically, cutaneous afferent input to the spinal cord via the superficial radial nerve appears to be presynaptically inhibited by motor commands thus reducing influence of the afferent input on the postsynaptic signal and this occurs in a concert with active wrist movements (Seki et al. 2003; for a review of receptors possibly involved in presynaptic inhibition see Miller 1998).

The attenuation of sensory inputs during a movement is understood mainly from the perspective of sensorimotor filtering, whereby the reafferent sensory signals are filtered out since they are produced by the animal itself and may interfere with the externally produced sensation (Bays and Wolpert 2007, Blakemore et al. 2000, Crapse and Sommer 2008, Cullen 2004). In this context, it is by definition important to selectively filter out only the self-induced sensation and leave the externally generated sensation largely unchanged. This may be accomplished by generating internal prediction of the sensation that is going to result from the upcoming movement and this prediction is formed on the basis of the motor command (von Holst & Mittelstaedt 1950, von Holst 1954, Sperry 1950, Jordan & Rumelhart 1992,
Wolpert and Miall 1996, Davidson and Wolpert 2005). Then, the predicted sensation may be subtracted from the actually experienced sensation upon the movement. Documented related ideas actually date back to Helmholtz and moreover to Descartes and his ‘Traité de l’homme’ (Helmoltz 1866, Descart 1664).

Importantly, it is plausible that sensory inputs are being both synergistically combined with the motor commands and, as described above, attenuated. These two seemingly opposite processing paradigms may have distinct neuronal substrates. The later paradigm involves neurons suppressed during the self induced sensation, putatively because of the inhibitory signals related to the corollary discharge and they respond fully only in the absence of the relevant movement (Bays and Wolpert 2007, Blakemore et al 2000, Crapse and Sommer 2008, Cullen 2004). The former population is however enhanced in the context of the movement. We propose that contact neurons may present a neuronal substrate for this type of sensorimotor function, having sensation enhanced specifically for a goal-directed action. A goal-directed contact is different from others because the animal intends to accomplish it and actually one of main goals of the movement is to acquire this sensation. We put forth two possibilities for this phenomenon and elaborate on neuronal substrates of such proposed phenomenon.

**Gating of Sensory Inputs**

The first possibility involves gating of sensory inputs. Importantly, such a gating of the sensory input resembles cortical gating of the motor output, which is one of the critical mechanisms in the motor system for preventing unwanted movements (Hikosaka et al 2000, Grillner et al 2007). Here, we propose that a mechanism preventing ‘unwanted’ sensations may be reflected at the level of the SC. This mechanism would have a general, important function in the sensorimotor system. In lower vertebrates the optic tectum, a homologue of the SC in non mammals, can
govern locomotor behavior as evidenced by locomotor movements evoked by electrical microstimulation in the optic tectum of the lamprey (Grillner et al 2007, Saitoh et al 2007). In addition, the SC is acknowledged as a pivotal structure for gaze orienting movements (Munoz 2002, Hikosaka et al 2000, Hall & Moschovakis 2004) and one of main features of the oculomotor circuitry is inhibition exerted over the SC by the substantia nigra pars reticulata (Hikosaka and Wurtz 1983b, Hikosaka et al 2000, Munoz 2002, Hall & Moschovakis 2004). This inhibition is thought to prevent unwanted eye movements – an eye movement can be executed only upon a removal of this inhibition. The substantia nigra pars reticulata receives inhibitory projection from the caudate nucleus, which is in turn strongly influenced by the prefrontal cortex (Hikosaka et al 2000, Munoz 2002). In this way, the cortex exerts control over the movement by exciting the caudate nucleus, which in turn inhibits the substantia nigra pars reticulata thus removing the inhibition of the SC and allowing the movement (Hikosaka et al 2000, Munoz 2002). This type of a pathway evidenced in monkeys within the oculomotor circuitry appears to present a general concept employed in vertebrates for the control of movements (Grillner 2007). Namely, it appears that the basal ganglia nuclei that inhibit the optic tectum receive an inhibitory input from the striatum. The striatal neurons projecting to the basal ganglia have zero baseline activity and specific membrane properties involving $K^+$ inward currents that dynamically drive the neuron away from the threshold for producing action potentials and make these neurons difficult to activate. They are though to be activated by the very strong signal originating from the cortex or the thalamus and strongly modulated by dopaminergic neurons (Grillner 2007). Therefore, via the striatum and the optic tectum or the SC, a prevention of the unwanted motor programs is actualized. Contact neurons of the SC may represent a neuronal substrate for a similar concept: prevention of the unwanted sensation. Namely, they appear activated only within a
specific context i.e. goal-directed movement and otherwise seem not responsive. In
the absence of the movement tactile sensation is prevented and allowed only within
the intended contact. This seems plausible because as mentioned this sensation
following the movement is actually a part of the movement goal. The remaining
question is the identity of the underlying neuronal pathway mediating sensory gating.
Since sensation and movement appear profoundly interconnected, the structures
involved in the motor gating may be at least in part functional for the sensory gating.
Thus, the established pathway from the prefrontal cortex via the striatum and the
basal ganglia that gates motor programs may be one possible candidate. However, in
the case of contact neurons the pathway should include structures involved in both
somatosensory and arm movement processing. Since corticostriatal input involves
different cortical areas that project mainly from the layer 5 to medium spiny neurons,
the striatal involvement seems plausible (Bolam et al 2006).

Enhancement by a Motor Signal

The second possibility is that this enhancement is putatively driven by the
motor related signal, whereby contact neurons integrate both somatosensory input
signals and motor signals. Motor signals depolarize the neuron, making it more
sensitive for the upcoming sensory input. Immediately upon the contact, sensory
input causes depolarization above the threshold and the neuron starts to be active.
Such gating of the neuronal output by the motor signal assumes that terminals of
axons that carry motor related signal exert strong influence over the neuron.
Importantly, it has to be difficult to cause the postsynaptic potential of the neuron to
reach the threshold without the motor related signal. Such influence can rely on a
high number of terminals from axons delivering the motor signal in which case the
motor signal would exert influence as described above. Another possibility is that
membrane properties of the neuron make it very difficult to activate because of the
membrane properties e.g. inward rectification by K+ currents as evidenced for instance in striatal neurons (Grillner 2007). This possibility may provide an explanation for neuronal activity of some contact neurons that starts in advance of the contact (Figure 7).

Neuronal Activity in Advance of the Contact

If the above described influence is strong, i.e. motor signals depolarize the neurons sufficiently, then the neuron can start to be active even in advance of the contact. This may happen for two reasons. First is that for the neuron close to the threshold probability to emit action potentials is high. The second reason may involve additional mechanisms related to the monitoring of the movement. Namely, the SC is involved in goal-directed behavior and as such may have access to the signals monitoring the progress of the movement towards the goal. This monitoring presumably relies on both sensory feedback signals e.g. based on the visual observation of the movement; and on internal forward modeling of the movement. In the case of quick movements such as under constraints of short reaction time, the monitoring relies more on the internal modeling. While the hand is approaching the target, the motor error i.e. the difference between the desired position of the hand on the target and the current position, decreases. Let us assume that the monitoring of this arm movement relies on the internal model. Decreasing the motor error increases the probability for the neuron to produce action potentials. When the error is small enough, the neuron may start to be active even before the contact actually occurs.

Finally, the output signal of contact neurons, specifically those active in advance of the contact, may directly relate to the prediction of the sensory consequence of the movement. The self-induced sensation (e.g. tactile stimulation upon the self-generated contact) can be predicted on the basis of the motor program.
(e.g. to move the arm and accomplish the contact). Such mechanism has been extensively discussed putting forth the cerebellum as one of the candidates that may form sensory predictions (Miall et al 1993, Wolpert & Kawato 1998, Bays & Wolpert 2007, Ito 2008). Here, the signal that excites contact neurons may originate from any putative brain structure that forms predictions including the cerebellum, which is interconnected with the SC via direct projections (Gonzalo-Ruiz et al. 1988; Gonzalo-Ruiz and Leichnetz 1990; May et al. 1990; May 2006). Another possibility is that the prediction is formed directly within the SC. In such a case these neurons may present a residue of the evolutionary old system from the time when the optic tectum played an even more pronounced role in behavior. In primates, large cortical expansion may have provided other additional structures for this type of processing. For the structure that governs goal directed behavior, however, it is necessary to keep record of a motor program and internal models that include prediction of the sensation that is going to result from the motor program. This may help to detect external stimuli occurring during the motion and to react accordingly. For the optic tectum as such structure in lower vertebrates, this system may have been embedded within putative neuronal types some of which may be similar to the one we report. In primates, the function of this system may be mainly overtaken by the cortex, however the residue may still echo in the preserved neuronal type.

**Putative Projections to Hand Muscles**

Alternative explanation for the neuronal activity occurring before the contact may be that they present a motor signal involved in the contact event e.g. related to the specific hand posture accomplishment. Arm movement neurons start to be active before and during the arm movement towards the target and those at lower depths typically have prelude activity as reflecting motor preparation (Werner 1993, Werner et al 1997, Lünenburger et al 2001, Stuphorn et al 2000). Contrary to them, contact
neurons are not active during the whole period of the hand approaching the target and start with the spiking immediately before the contact (e.g. Figure 6). However, hand muscles possibly participating in the last stage of the movement immediately before the contact event may be recipients of such a motor signal communicated by the SC. This notion would be supported by unpublished anatomical observations of Rathelot, Strick and colleagues who found polysynaptic connection from the SC to finger muscles in a rhesus monkey after retrograde labeling using transneuronal virus transport technique (Strick PL, personal communication). In this case, the neuronal activity of contact neurons would be rendered as a motor signal for finger and hand muscles. In our knowledge, SC neurons providing such a signal have never been described. On the other hand, it is unlikely that putative hand muscles are not active at all during the whole sequence of motor events contained in the task. One would expect that motor signals would exhibit at least some presence during the task and not exclusively for the contact event.

**Contact Neurons Relationship with the Contact Force**

Contact neurons appear mainly not influenced by the magnitude of force exerted during the contact. Correlation of the peak force with the number of spikes produced by a neuron around the contact time was significant for two out of six neurons that had force measurement available and for both the correlation was moderate. Here the initial activation of the neuron immediately upon the contact was examined, before the perturbation occurred on perturbed trials, and therefore the result applies for both unperturbed and perturbed trials. One of the two significant correlations was negative and the other positive suggesting different effect the force may have produced. If contact neurons were simply somatosensory the force magnitude would have a stronger effect on their activation.
Furthermore, cross-correlation between the smoothed instantaneous spiking frequency function of contact neurons and the contact force on a trial by trial basis for unperturbed trials results in moderate correlation (Figure 11). The same analysis for perturbed trials results as well in moderate correlation however higher than on unperturbed trials (Figure 12). However, there was a significant difference between correlations on perturbed vs. unperturbed trials on a trial by trial basis ($p=2.4660\cdot10^{-10}$, Two-way ANOVA) and cross-correlation of super arrays was higher for perturbed than for unperturbed trials ($p=0.0062$, Two-sample T test; Figure 19). This may have important implication for understanding the origin of contact neurons’ neuronal activation. In perturbed trials there is an additional component of force originating from the external event of perturbation. This external force is the only difference in force between the two trial types. Since, the difference in correlation is highly significant it suggests that this external force component may underlie higher correlation of neuronal activity with force on perturbed trials. Therefore, contact neurons may be particularly sensitive to external force that occurs during self-generated contact.

In this context, we may reexamine whether the activation of contact neurons starting around the time of contact or immediately before may carry the signal about the predicted sensory consequence of the goal-directed movement. Let us consider unperturbed trials since they comprise only self-generated contact force. If contact neurons signal the predicted sensation resulting form the movement their activity would be expected to correlate with the applied force since this force depends on the motor program. However, the observed correlation between the neuronal activity and the contact force is low for peak neuronal activity vs. peak contact force, and for number of spikes around the contact vs. peak contact (Table 4). On the other hand, cross-correlation between the neuronal activity and the contact force as time
functions results in moderate correlation both for trial by trial cross-correlation and for cross-correlation on super arrays (Figures 11, 12, and Figures 16, 18). These observations may not be conclusive because the subject’s behavior emerged to be stereotypical in the task i.e. the contact force was similar between trials. Fluctuations in the neuronal activity and the contact force thus may not have been sufficient for a conclusive correlational analysis. We propose that the question whether contact neurons signal predicted sensation may be addressed in another experimental design. Such a design would require two different motor programs with two different force levels. One trial would comprise a precue that instructs strong or light contact (e.g. a target button lit red means strong contact and yellow light). Then upon a go signal is given everything would be the same except the internal motor program of the subject. If contact neurons communicate sensory prediction signal then their activity would be different for the different motor programs. Our current experimental design appears to leave open the question about possibility that the neuronal activity of contact neurons relates to sensory prediction.

**Influence of Perturbation on Contact Neurons**

Upon perturbation, some contact neurons were responsive by either increasing (4 out of 8 neurons) or decreasing (1 out of 8 neurons) total number of spikes in a trial (Table 3). On the other hand, for some contact neurons (3 out of 8) total number of spikes did not significantly differ between perturbed and unperturbed trials. As examined above, the cross-correlation between the smoothed instantaneous spiking rate and the contact force revealed higher perturbation on perturbed trials suggesting that at the level of population the perturbation exerted a significant effect.

In the context of the sensory gating proposition, whereby contact neurons are inhibited for ‘unwanted’ sensations i.e. those that occur outside of the goal-directed
arm movement towards the target, their neuronal activity should not be affected by perturbation. Namely, the perturbation carries external sensation that would be suppressed in this context. Therefore, only neurons that do not significantly change their activation in perturbed trials relative to unperturbed may conform to such proposition. However, the sensory gating may be exerted over the neuron during the whole contact time that would then include the perturbation event as well. Thus, a contact neuron is then responsive to sensory stimuli over the whole course of the contact and the perturbation is an effective stimulus.

Another described idea stated that motor signals exert strong influence over the contact neuron and the neuron is sufficiently sensitive to sensory input only if brought closer to the firing threshold by this motor related input. In this context, it is disputable how long the neuron stays sensitive upon the completion of the motor program. If the sensitivity is prolonged over the contact period the neuron may be responsive to the perturbation. Fluctuation of the time period of this sensitivity may underlie different response magnitudes to perturbation observed in contact neurons.

**Perturbation Neurons**

Introduction of perturbation in the task revealed neurons activated almost exclusively in perturbed trials (Figures 27, 28). Here we propose one interpretation for the observed neuronal activity. In unperturbed trials sensation the subject experiences originates from the self generated motor program. Therefore, it can be anticipated on the basis of the motor program. The anticipatory signal may come from the SC itself (e.g. presumably via the signal from contact neurons) or it may be provided by other brain structures. In perturbed trials, however, overall sensation that the subject experiences is not solely a consequence of his own motor action but contains one additional component caused by the perturbation. The discrepancy between the predicted and the actually experienced sensation may be the stimulus
for perturbation neurons. Such proposal and its counter arguments are discussed in following sections.

**Possible Influence of the Cerebellum**

The range of acknowledged possible functions of the cerebellum has been expanding in addition to its established sensorimotor roles (for a review see Strick et al. 2010, Timmann and Daum 2007, Glickstein 2007). Amongst other, the cerebellum has been acknowledged as a part of the system that predicts sensory consequences of intended movements (Ito 1970, Miall et al. 1993, Wolpert & Kawato 1998, Bays & Wolpert 2007). If perturbation neurons participate in discerning between predicted and actually experienced sensation upon the movement then the putative external structure providing such sensory prediction signal may be the cerebellum. In addition, the long neuronal latency of some perturbation neurons suggests that the neuronal signal may emerge within multiple neuronal loops or that it propagates through a longer polysynaptic pathway. There is an ample anatomical evidence that the SC and the cerebellum are strongly interconnected, whereby mainly inputs from the cerebellum to the SC have been described (Gonzalo-Ruiz et al. 1988; Gonzalo-Ruiz and Leichnetz 1990; May et al. 1990; May 2006). There are two main so far documented routes of this connection. The first comprises axons originating in the posterior interposed nucleus and nearby dentate nuclei and terminating in the contralateral SGI while the second comprises axons originating in the fastigial nucleus and terminating in the rostral poles of both colliculi with a contralateral predominance (Gonzalo-Ruiz et al. 1988; Gonzalo-Ruiz and Leichnetz 1990; May et al. 1990; May 2006). Both neuronal classes active in our task contact and perturbation neurons we found almost exclusively in the lateral SC zone and they did not tend to cluster in the rostral pole of the SC. Therefore, it appears that the first route is a more likely candidate for the cerebellum to influence perturbation neurons.
On the other hand, the putative connection between the SC and the cerebellum may be via previously not described routes featuring closed loop architecture. Namely, one of main features of the connections the cerebellum has with the cerebral cortex is the presence of anatomical loops: if the cortical area receives projections from the cerebellum then it projects back to the cerebellum via the pons (Strick et al. 2009, Kelly & Strick 2003). This principle was evidenced using retrograde transneuronal transport of rabies virus suggesting projections from Purkinje cells within lobules IV-VI of the cerebellar cortex to the arm area of the primary motor cortex and using anterograde transneuronal transport of a herpes virus suggesting projections from the arm area of the primary motor cortex to granule cells within lobules IV-VI (Kelly and Strick 2003). Similar organization was shown for connections between area 46 of the prefrontal cortex and Crus II of the ansiform lobule within the cerebellar cortex (Strick et al 2009, Kelly and Strick 2003). Thus, closed loops appear to present important feature of the connections the cerebellum forms with the cortex. In this context, it may be plausible that the connections between the cerebellum and the SC are similarly organized featuring closed loops as anatomical and functional architectural units. If so, the signal emerging at the output of perturbation neurons may originate from a putative tectocerebellar loop. This could be an explanation for a very long neuronal latency observed in some perturbation neurons. The remaining question would be the exact pathway connecting the SC and the cerebellum. In cerebro-cerebellar loops pathways include pontine nuclei and the thalamus: the cortical area projects to the defined region of the pontine nuclei, and this region projects to the cerebellar cortex; at the same time, this region of the cerebellar cortex projects to the specific part of the dentate nucleus of the cerebellum, which then projects to the specific region in thalamus, and finally this region of the thalamus projects to the cortical area. The putative tectocerebellar loop may not include the
thalamus since it appears mainly reserved for the signals reaching the cerebral cortex (e.g. Bear et al 2001).

**Are Perturbation Neurons Multisensory Neurons?**
The observed response of perturbation neurons may not be related to higher sensorimotor functions as we proposed but may simply reflect a sensory response to the stimuli contained in perturbation. This alternative interpretation seems probable especially due to the response of tested perturbation neurons in the control tasks, whereby the subject was passively exposed to the audiovisual features of the perturbation event. Thus one may hypothesize that perturbation neurons actually present population of multisensory neurons in the SC. Multisensory neurons in the SC have been previously described in the cat and in the monkey and they present a main neuronal substrate for the phenomenon of multisensory integration (for a review see Stein & Stanford 2008, Stein & Meredith 1993). Such neurons respond to stimuli from more than one modality (e.g. to both audio and visual stimuli) and to their combinations. Furthermore, their response to the combination of stimuli from different modalities can be higher than to the most effective of those stimuli alone or moreover than the sum of responses to stimuli from each modality alone (e.g. number of spikes in response to auditory and visual stimuli presented together is higher than sum of numbers of spikes in response to audio and visual stimulus alone). This phenomenon is denominated as superadditivity (Stanford & Stein 2007). Multisensory integration has been extensively studied from the gaze-orienting perspective establishing that receptive fields of multisensory neurons for individual modalities are aligned (Jay & Sparks 1987, Wallace et al 1996). This aids detection of a single event since sensory signals originating from one event originate from the same location. There are a number of reasons rendering the hypothesis that perturbation neurons simply present multisensory neurons actually implausible.
First, perturbation neurons do not respond to task-irrelevant sensory stimuli. They were tested with a variety of task-irrelevant auditory stimuli including broadband sound bursts that resembled the perturbation sound. It can be argued that such stimuli were not optimal for a given neuron however they should have elicited some response albeit weaker than for the assumed optimal sound of the perturbation, which was not the case. On the other hand, some multisensory neurons in the monkey described by Wallace and colleagues are vigorously active only upon presentation of combined stimuli while responses to unimodal stimuli can be extremely weak (Wallace et al 1996). Such neurons illustrate the principle of superadditivity. This leaves the possibility that testing separately with auditory stimuli was not sufficient to elicit detectable response. However, in our Control task 2 the subject was exposed to the sound of perturbation which elicited neuronal responses. Therefore, the tested neurons did not belong to the group of multisensory neurons that respond very weakly to unimodal stimuli. As such they should have responded to task-irrelevant auditory stimuli, which was not the case.

In addition, tested neurons were exposed to task-irrelevant visual stimuli including those resembling the panel motion such as moving the board of a similar size, which did not elicit responses. The only effective visual stimulus was the motion of the working panel itself suggesting that the observed response may have emerged due to association. We do not exclude the possibility that these neurons represent some very specialized previously unreported sensory neurons that respond to stimuli exactly contained in the perturbation event although it would be difficult to conceptualize their responses in the context of the current knowledge about the sensorimotor system.

Moreover, the observed neuronal latency of perturbation neurons is longer than the latency of known multisensory neurons in the SC. Mean auditory neuronal
latencies for multisensory neurons were 18 ms in anesthetized preparation (Wallace et al 1996). In the behaving monkey Jay and Sparks found median auditory latency 44.8 ms while having almost all neurons with auditory latency below 80 ms (Jay & Sparks 1987). All these values are highly exceeded in perturbation neurons (Figure 33). Three perturbation neurons with lowest neuronal latencies may possibly conform to the longest latencies Jay and Sparks reported.

Notably, activity of some perturbation neurons (5 out of 10) outlasts the perturbation event for more than 500ms i.e. action potentials are produced after the panel motion ended. Such prolonged activation appears unusual for the simple sensory responses.

Finally, the responses of perturbation neurons in Control task 1 and 2 may have occurred due to association. Neurons tested in these control tasks were collected at the time when the subject had extensive experience in the task. Figure 33 shows neurons in the chronological order from left to right. Thus, neurons at the late stage have auditory response that may have emerged due to association since the perturbation sound was always present in the perturbation event. This additional auditory response causes the neuronal latency to be effectively shorter (note a separate mode in the task response, Figure 34). On the other hand, distinct neuronal classes may be present within the group of perturbation neurons we observed. The neurons with the longest latencies may be the class that detects the discrepancy of the predicted and the experienced sensation caused by a movement and their signal may originate in more complex sensorimotor processing possibly involving multiple loops in the brain.

Contact and Perturbation Neurons as a Putative Part of One Network

Reported neuronal classes of contact and perturbation neurons may be interpreted as a part of one sensorimotor network involved in goal-directed arm
movements. Here we summarize discussed interpretations in the context of the current knowledge about the sensorimotor system. Before every movement, a neural processing amounts in an appropriate motor command signal issued by the motor structures in the brain (Figure 53). At the same time, this signal is sent as an efference copy to a predictor system in the brain that forms the expected sensory consequence of the upcoming movement. The actually experienced sensation upon the movement is then compared with the predicted one and the result of this comparison is the discrepancy signal. For a self-generated action the predicted sensation is close to the experienced one and the discrepancy signal is close to zero. In our task this is the case during unperturbed trials. During perturbed trials however, the panel unexpectedly moves and produces additional sensory input that cannot be predicted on the basis of the motor program. The overall sensation is then different from the expected one and, the discrepancy signal is substantially different from zero. This discrepancy could be an effective stimulus for perturbation neurons suggesting their activity as a neural substrate for signaling an external event. On the other hand, the neurons that start to be active immediately before the contact may present a part of the predictor system in the brain that forms a signal corresponding to the predicted sensory consequence. Thus, together with population neurons they may participate in the system that differentiates between self-induced and external events.
Figure 45. *Sensorimotor Processing Signal Flow.*

A motor command via the motor system produces a specific movement. The same signal as a so-called efference copy, is sent to the predictor system in the brain. It outputs the sensation signal that is expected to result from the upcoming movement (predicted sensory consequence). On the other hand, the movement execution has its sensory consequence (the central signal flow). In the absence of the external event the overall sensation equals this consequence. Then, for the accurate prediction of the sensory consequence, the discrepancy signal is close to zero. In the presence of the external event however, the overall sensation is the sum of the sensory input produced by the external event and movement’s sensory consequence. In that case, the overall sensation significantly differs from the predicted sensory consequence and this provides the discrepancy signal. This discrepancy signal informs that an external event occurred.
This notion is supported by evidence that the SC is functionally involved in goal-directed behavior in rat (Felsen and Meinen 2008). In addition, the SC may even play an important role in the higher level goal-directed behavior in primates. Namely, Dorris and colleagues used an oculomotor version of a simple strategic game whereby monkeys were free to choose one of the two targets while competing against the computer opponent (Thevarajah et al 2009). They showed that the activity within the intermediate SC layers was predictive of an upcoming eye movement in the game. In addition, the predictive power gradually increased over time preceding the eventual eye movement suggesting that motor representation of available options competed at the level of the SC. This has important implications for understanding the SC role in such a process being consistent with the SC integrating cognitive signals about the decision where to go (in this case where to move the eyes) and resolving them within its neuronal circuits leading to an eventual eye movement. Thus, the SC appears not to be a passive center performing only low level processing to prepare an eye movement prespecified by other structures. In addition, subthreshold electrical microstimulation (that did not elicit eye movements) within the intermediate SC layers biased monkeys to select the target in the zone of the visual field corresponding to the stimulation site more often. Without the stimulation monkeys behaved close to the game theoretic equilibrium – their choices were equally distributed between the options (one target 50% of time) and almost stochastic so the computer opponent was not able to exploit predictable patterns in their behavior in a larger extent (in this way monkeys maximized reward intake). Thus, the primate SC seems to be functionally involved in higher cognitive phenomena such as motor program selection under strategic conditions. If so, the SC may be a plausible brain structure even in primates to contain neurons showing a higher level processing despite cortical enlargement.
Finally, anatomical evidence about SC connections with the motor and premotor cortices and sensory areas may support the notion about its proposed sensorimotor role (May 2006, Fries 1984, 1985). In addition, a very recent anatomical study described one additional input that the SC receives from motor structures, a projection from the hand field of the ventral premotor area F5 (Borra et al 2010). Area F5 is known for containing so called ‘mirror’ neurons, which are active for both a performance of a specific action by monkey and observation of the same action performed by someone else (for a recent review see Rizzolatti & Sinigaglia 2010). The function of mirror neurons is not fully understood and one of the interpretations suggests their role in action understanding. The SC zone targeted by the projections from the area F5 seems to overlap with the zone where contact and perturbation neurons are found (lateral zone of the SC) (Borra et al 2010; Elena Borra, personal communication).
METHODS

Apparatus
A subject was seated in the custom made Plexiglas primate chair surrounded by a magnetic field system for eye position monitoring (Primelec, D. Florin) embedded in a black wooden frame (70 cm x 70 cm x 70 cm), which was fixed on a base support construction made of aluminum (H x W x D: 90 cm x 70 cm x 70 cm). Analog signals corresponding to the horizontal and vertical eye position were sampled with the 500 Hz sampling rate. A vertical working panel mounted on its own aluminum construction was placed in front of the subject 31 cm away from his eyes and fixed to the wooden frame. The working panel comprised two parallel plates made of Plexiglas, connected via four plastic cylindrical holders (1 cm diameter, 5.15 cm length). The proximal plate (the plate closer to the subject) had dimensions 40 cm x 52 cm x 0.5 cm (Height x Width x Thickness), the distal plate 40 cm x 56 cm x 1.2 cm (Height x Width x Thickness) and the distance between them was 5.15 cm. Between the two plates, a force sensor (KD40S, 100N, ME-Meßsysteme) was fixed. The sensor itself rested on the distal (thicker) plate and was connected to the proximal plate via a metal rod that perpendicularly touched the proximal plate 27 mm below the central target button position (relative to the button centre). In this way, the force applied on the proximal plate was transmitted to the sensor. This design facilitated detection of a total force applied on the panel during the contact and not only the fraction exerted on the target button (since subject’s hand was bigger than the button). The proximal plate of the working panel was covered by a white translucent sheet. Five buttons (RAFI 1.20 122 ENEC 10) were mounted on the proximal plate with one button in the centre and four others symmetrically distributed forming a cross. Distance of all buttons from the central one was 8 cm. Each button comprised a dark plastic base with diameter 23 mm, and thickness 7 mm, and white plastic
semi-translucent core that protruded 3 mm from the core; thus, the thickness of the button was 10 mm when free and 7 mm when fully pressed. Each button contained three light-emitting diodes (red, green and blue) under the semi-translucent plastic surface. The diodes were under the control of the task computer via cables that were conducted between the two panel plates. The distal plate was at the lower edge connected to additional holders made of tough grey plastic, that were in turn connected to the aluminum construction via two cylinders to enable rotational freedom. Hence, the panel could rotate around the horizontal axis close to its lower edge.

The panel was connected to the custom made force perturbation system that could induce an abrupt panel motion upon subject’s contact with a panel button (Figure 46). The perturbation system was pneumatic and used compressed air from the in-built university system in the building. Compressed air first entered pressure regulator (FESTO MS4-LFR-1/8-D7-ERM-AS, 381035 V3, 529172). At the beginning of each session the air pressure was adjusted to be 5 Bar. This value was chosen to assure a constant pressure throughout the session since higher values were close to the maximal value the university network could provide and these might have undergone value fluctuations. The air output of the pressure regulator was directed to the electronically controlled valves within an interface controller box (FESTO CPE 10-M1BH-5L-QS-6) attached to the Pentium 1 task computer. The air output of the valve box was via plastic tubes (FESTO PUN, H 6X1) connected to the cylinders that moved the panel. The computer triggered perturbation by sending two pulses to the valve box controller. The first and the second pulse initiated the first phase of the panel motion (e.g. towards the subject) and the second phase of the panel motion (e.g. away from the subject), respectively. The time interval between the pulses was controllable by the computer user. By increasing this interval the duration and the
amplitude of the panel motion were increased. The panel motion depended as well on the subject’s contact force in a given trial. The cylinders that moved the working panel were on one side attached to the panel and on the other side to the aluminum construction. The point of attachment on the panel was immediately below the upper panel edge in the middle of its width. The distance from this point to the axis of panel rotation was 42.5 cm. Appropriate free motion of the cylinders and the panel was assured by custom-made ball joints.

![Diagram](image_url)

**Figure 46.** Scheme of the Experimental Setup with the Perturbation System (Side View of the Working Panel)

Upon the contact, the target button is switched on and this is registered by the computer, which in turn sends the trigger signal to the pneumatic actuator, which moves the panel (induces its brief reversible displacement by a rotational movement around the bottom axis).

**Visual Stimuli**

The room luminance during the session was below 0.1 Cd/m². A green, rectangular fixation point used in the task had a luminance 0.2 Cd/m², and dimensions 4 mm x 4
mm. The stimuli were back projected on the white translucent sheet mounted on the back side of the front plate of the working panel via a digital projector. Optical filter (SCHOTT, 0.0025, Type NG9, Schmelze 347831, ø 50 mm, Thickness 1.81 mm) was used at the objective of the digital projector in order to reduce the luminance of the background image. Target buttons on the working panel, after embedded illumination was switched on by the computer, had 0.7 Cd/m² luminance if red (i.e. target precue), and 1 Cd/m² luminance if green (i.e. ‘go signal’).

Arm Movement Task

To initiate a trial the subject was required to put his hand on the start button mounted on the horizontal Plexiglas plate in the level of the subject’s hip. This triggered an appearance of the fixation point on the vertical working panel and the subject was required to visually fixate the point. After a random delay (usually set between 50 ms and 200 ms) following the onset of fixation, the central button lit red as a precue and after another random delay (usually set between 200 ms and 300 ms) green as a ‘go’ signal. Upon this instruction the subject moved his arm and reached for the target button. The subject had to start the arm movement within a specified time window after the ‘go’ signal. In the initial training stage a relatively quick reaction was required (the allowed time window was set below 500 ms) to assure that the subject learns to initiate the arm movement upon the ‘go’ signal. Later, this urgency was removed to create a less stringent task context for the subject since the reaction time was not important for the study (the allowed time window was 2000 ms). Nevertheless, the subject typically initiated the arm movement towards the target with a reaction time below 500 ms (this is the time when the computer registered the release of the start button micro-switch, therefore the actual movement initiation occurred even earlier since there is a significant inertia of the arm and a delay of the button switch). After pressing the target button and maintaining the
contact, the subject returned his hand to the start button. Initially, the task included a button color change from green to yellow after the contact as an additional ‘go signal’ for the arm movement back. However, this signal was not visible to the subject due to his strategy to press the button with the palm (while having fingers bended resembling a shape of a loose fist, so that he exposed the lower part of the palm and the dorsal side of the fingertips to the panel). Thus, the arm movement back was initiated by the subject without a direct instruction, however too long contact was not allowed by setting a specific time window for the back movement initiation. The subject had to initiate the movement back within a large time window (usually set between 2000 ms and 3000 ms) upon the contact to promote a reasonably long contact time, reduce urgency in performance and assure a natural task performance while still requiring alertness in the task. Upon returning his hand back to the start button, the subject had to continue with visual fixation for a brief period (usually set between 300 ms and 500 ms) after which he obtained a liquid reward. The visual fixation thus had to be maintained during the entire trial.

The described event sequence was also applied in the different version of the task with the inclusion of an additional peripheral reach. In this version, the subject first contacted the central target button and then after a brief randomized delay usually set between 300 ms and 500 ms one of the four peripheral buttons lit red. After a brief randomized delay usually set between 300 ms and 500 ms this button changed a color to green, which was a ‘go’ signal for the subject to release the contact from the central button and press this peripheral button. The rest of the trial comprised the previously described event sequence. This version was initially used and then replaced by the version that required only the central reach. This was done to remove the redundancy from the task, since the peripheral reach was not necessary for investigation of contact phenomena and, importantly, to simplify the
task for the subject. Namely, one of the main obstacles for the effective conductance of the study was subject’s working attitude. It appeared that each additional step in the task increased a probability that the subject would stop to perform after a low number of trials. Thus, every element that appeared not critical for the research goals was removed from the task.

**Reward Delivery**

The reward system contained the valve box with electronically controllable valves (Bürkert, 0330 C3.0 NBR MS) that was connected to the task computer. The task computer delivered a reward by sending a TTL pulse that opened the valves. The duration of the pulse determined the amount of a delivered liquid (juice or water). The reward liquid was situated in the plastic cylindrical container mounted approximately 2 m above the ground behind the subject on his left side (at the left, back corner of the magnetic enclosure). The container was connected to the input of the valve box via plastic tubes (ø 10 mm). The output of the valve box was connected to the aluminum sipper tube (ø 5 mm) via plastic tubes (ø 10 mm). The sipper tube was fixed on the primate chair immediately in front of the subject’s mouth so the reward could be comfortably acquired. Reward was either water or juice chosen from a variety of fruit juices. After testing orange, grape, apple, Bionade® Holunder, Bionade® Ingwer juices and variety of sweetened fruit and herbal teas (cooled to the room temperature) the choice eventually amounted to a black currant juice (25% fruit content) which appeared to be preferred by the subject and which was then used regularly. Occasionally, grape juice (100% fruit content) was used. The usual amount of reward during the session was approximately 0.5 ml per successful trial and was increased at the end of the laboratory session to additionally motivate the subject. The subject was free to obtain unlimited amount of reward during the task.
**Subject’s Habitat and Behavior**

The subject dwelt in the spacious room together with two to three other male rhesus monkeys. The habitat was enriched with one tree trunk hanging from the ceiling on two chains, one big tree branch with collateral branches mounted so to enable climbing from the half height room level to the ceiling, several shelves fixed on the walls above two meters height, and one swing made of wood hanging from the ceiling on the thick robe at height of one to one and half meter from the floor. In addition, a variety of toys was provided made mainly of tough plastic materials. The room had windows on the west side and entrance on the east side. It was separated in two parts by a big metal (zincified stainless steel) mesh construction that contained four cages and the narrow doors beside the cages as a connection between the two parts. One part of the divided room was smaller, situated at the entrance and equipped with a water sink and a weight scale. The bigger part of the room on the other side of the construction was a monkeys’ residence. Windows were protected by the same metal mesh used for cages; the lower part was opaque while the upper part above two meters of height was normally transparent so monkeys could see the sunset if resided on one of the upper shelves. The floor and walls were covered with light-grey ceramic panels.Occasionally, the big bunch of dry grass was provided with toys or suitable food such as dry fruits hidden inside, enabling foraging activities. The monkey cages were elevated approximately 0.7 m above the ground so that monkeys could comfortably use the space below the cages as well. They entered the cage through sliding doors situated at the bottom of the cage. These doors were controlled from the entrance part of the room so one could open and close them by pulling and pushing, respectively. Animal care takers used to clean the residence every morning. Before cleaning they assured that monkeys entered their cages. Usually, monkeys entered promptly upon the animal care takers vocalized and
unlocked the doors separating the monkey part from the entrance part of the room. If not, they placed a banana in the cage, which attracted a disobedient inhabitant to enter. Before the laboratory session, monkeys were situated in their cages so a safe acquiring procedure could be performed.

One of the main obstacles during the study was inappropriate working attitude of the subject. He tended to stop working after a low number of trials, very often even after the initial fixation task. We tried to determine reasons for such behavior. Upon monitoring out of the laboratory we noticed that subject's liquid intake was generally very low compared to other monkey subjects. Even when liquid was freely available in the habitat, his daily water intake was below 400 ml, and there were periods when this intake dropped almost to zero, without obvious reason. Thus, a liquid reward was apparently not motivating enough. We tried to additionally motivate the subject through intensified pleasant social interaction; this sometimes appeared to be the only mean to motivate him for work in the task. The interaction included different types of massage during the health check before each session mostly applied to the face and head region, delivery of fruits and favored food supplies (sweets based on natural ingredients and unsalted nuts), and importantly verbal communication. This communication comprised positive expressions as ‘good job, buddy’ upon him performing something appropriate, and the subject was responsive to it. His responses mostly included two types of vocalization. One was a high pitch calling-like sound and the other low pitch sound that expressed satisfaction or positive attitude (similar to the vocalization rhesus macaques produce before grooming).

**Acquiring the Subject from the Residence**

Prior to an experimental session, a subject was acquired from his residence and transferred to the laboratory. The subject was trained for the acquiring procedure, which comprised following steps. First, the researcher attached a collar
chain to the cage doors. Then, the subject entered the lower part of the cage upon the researcher took the separating plate and friendly communicated to him. This was followed by insertion of the separating plate, confining the subject to the lower part of the cage to enable safe, controlled acquiring procedure. Then the researcher took a blunt hook made of thick stainless steel wire and friendly communicated to the subject. Upon this, the subject came close to the cage doors enabling comfortable attachment of the collar to the collar chain. Then the collar chain was guided through the custom made stainless steel post, which comprised a circle on its both ends: the chain was put through the circle on one side, pulled to the other end of the post and attached to the circle on this end. The chain was held loose to prevent mechanical pressure on the subject. Then the cage door was opened and the subject was instructed to go to the weight scale situated a half meter away from the cage (by saying a word ‘Waage’, which is a German word for a weight scale). After measuring his weight, he was instructed to enter the primate chair, which was situated less than half a meter away from the weight scale (by saying a word ‘Stuhl’, which is a German word for a chair). Upon the subject entered the primate chair the neck plate was introduced followed by closing the doors of the chair. Then, the chain was detached from the collar and the subject was free to move albeit the motion was restricted mainly to rotation around the vertical axis since the neck plate prevented the head from being pulled inside the chair and prevented the body from exiting the chair enclosure. The subject was transported to the laboratory, situated less than eight meters away from the subject’s residence, by using a platform equipped with wheels, on which the primate chair was placed.

**Preparation for the Recording**

At the beginning of each laboratory session the subject’s health state was controlled and standard cleaning procedure was accomplished. The head was fixed
by a custom made titanium receptacle and this procedure was exercised in a way comfortable for the subject, involving his active participation. It was immediately followed by pleasant social interaction and a massage of a face and head region favored by the subject. The recording chamber was first rinsed with Ringer solution under moderately strong fluid flow via a plastic infusion tube, while having the 500 ml infusion bottle with the solution higher than 2 m above the ground. The circulated fluid was simultaneously aspirated via a sterilized glass pipette connected to the vacuum pump via plastic tubes. Then, 1% Hydrogen Peroxidase was injected into the chamber in the amount that covered the exposed tissue. The fluid was then mixed with a sterile Q-tip by performing circular movements without touching the tissue, until obtaining rich foam (usually after less than ten seconds). The foam was then aspirated and the chamber rinsed again with the Ringer solution while simultaneously aspirating the circulated fluid. The Peroxidase was then applied again and mixed in the described manner. The whole procedure was repeated until the foam formation was absent (usually two iterations were sufficient). The absence of rich foam indicated absence of organic debris in the chamber. At the end, the chamber was once again rinsed with the Ringer solution while simultaneously aspirating the circulated fluid and then a local anesthetic (Conjucain EDO, Bausch & Lomb, active substance Oxybuprocainhydrochlorid 4mg/ml) was applied. Occasionally (less than once a week), the chamber was filled with Iodine solution for five minutes before the last rinsing. At the end of the described procedure, the chamber was closed to be opened again only immediately prior to recording, which followed within less than ten minutes. The surface of the exposed tissue was 227 mm² (corresponding to the 17 mm inner diameter of the recording chamber) and the estimated depth of the wild tissue formed on the top of the dura was between two and three millimeters. Thus, the anesthetic was left for the prolonged period of time (between application and
recording, which was less than ten minutes) to saturate the exposed tissue and attempt safe anesthetization of the penetration zone including the dura. After the cleaning procedure the subject’s head was made unrestrained and he was placed with the chair in the recording construction. The chair was fixed to the aluminum base and the enclosure with the eye-position system coils was placed over the chair. Then the head was restrained again in the described way and the eye-recording paired lid was plugged into the eye-position contact embedded in the dental acrylic, anterior to the head holder. This contact was connected to the implanted scleral eye-coil. The recording chamber was then opened and the excessive amount of anesthetic removed by carefully contacting the lowest part of the chamber once with a sterile q-tip. The recording micromanipulator (Narishige MO-97) was mounted on the chamber and fixed by adjusting three chamber screws. The guiding tube was then carefully inserted by rotating the micromanipulator guide tube holder position knob. The guide tube position was monitored on the micromanipulator’s scale and additionally inspected through the transparent window of the micromanipulator. The contact of the guide tube with tissue was indicated by deflection of the tissue surface and was observable via a change in light reflected from the surface. From this point, the guide tube was advanced until the dura penetrated and then it was stopped. The dura penetration was detected by a characteristic, abrupt change in mechanical resistance to the guide tube. Current approach in neurophysiological recordings within deep brain structures is to penetrate with the guide tube deeply in the brain in order to closely approach the targeted structure; then the electrode is advanced until the targeted structure is eventually reached. However, we decided to stop the guide tube immediately upon the dura penetration and to advance through the brain only with the electrode in order to minimize the tissue damage since the guide tube diameter is approximately ten times bigger than that of the electrode, and its cross-section has
approximately hundred times bigger surface. In addition, the tip of the electrode is extremely sharp and it can pass cells and other tissue objects allowing the remaining part of the electrode shaft to push them aside without damage. At the same time, the electrode appeared safe for tissue objects such as blood vessels in the case of a direct impact (described below).

**Electrodes**

Initially, we used custom made glass-coated electrodes (hyperbolic shape, 1MΩ impedance at 1 KHz, produced by Hermann Korbmacher, Department of General Zoology and Neurobiology, Ruhr-University Bochum). Although providing excellent electrical signal, they appeared mechanically sensitive, especially their tip and we switched to another electrode type. All reported neurons were recorded with plastic coated electrodes with a total diameter of 110 µm or 120 µm (110 µm total diameter: 100µm Tungsten core, 10µm Narylene coating, 30 degrees tip angle; 120 µm total diameter: 100µm Tungsten core, 10µm Narylene coating, 30 degrees tip angle; NanoBiosensors, a new company name is WeSense). We tested electrodes of 60 µm total diameter (50 µm Tungsten core, 5 µm Narylene coating, 30 degrees tip angle, NanoBiosensors), as well of 70 µm diameter (60 µm Tungsten core, 5 µm Narylene coating, 30 degrees tip angle, NanoBiosensors), however they were not mechanically firm enough to penetrate the tissue – they bended upon entering the tissue. Namely, we were able to monitor the part of the electrode that was out of the guide tube. If the electrode faced an obstacle (such as when trying to penetrate the dura) its outer part between the entrance of the guide tube and the point of fixation to the electrode holder started to bend. At the beginning of each penetration we carefully monitored the state of the electrode. If the outer part started to bend that was a sign that the dura was not penetrated by the guide tube. Then, we advanced the guide tube slightly deeper according to the feeling mostly relying on the estimate
of the mechanical resistance to the guide tube motion. After this readjustment the electrode was advanced again. Before and after each experimental session we inspected the electrode under a microscope. Particularly important was to examine whether the tip of the electrode was damaged after the penetration. The described procedure with penetrating the dura proved to be safe for the electrode and in no single case it produced damage. However, we would emphasize that such a procedure may be risky for glass-coated or other types of electrodes. In addition, we monitored the state of the electrode above the guide tube during the recording. In some cases it started to bend while the electrode tip being already deep in the brain. We believe this was an indication that the electrode tip faced an obstacle such as a blood vessel that could not be penetrated. Importantly, this may suggest that our approach was safe for blood vessels and we speculate that our approach was generally safer than standard approaches that involve usage of guide tubes or thick electrodes.

**Typical Course of the Recording**

After the described preparation the main room illumination was turned off while still having one small light source providing low to normal visibility. The electrode was then advanced by the hydraulic micromanipulator and moved through the cortex until reaching the ventricle above the midbrain. Entering the ventricle was detected by a characteristic decrease in the recorded signal (so called ‘silence’). The electrode was then moved very slowly and the signal carefully monitored both on the oscilloscope and via the speaker. Approaching the superior colliculus was indicated by subtle however audible modulation of the signal during saccades. This modulation is thought to originate from visual stimulation of the SC superficial layers. This signal modulation was first subtle and then strong with relatively abrupt transition. Upon having the first signs of clear neuronal activity (characteristic sound of neuronal firing
and spike shapes on the oscilloscope) the electrode was stopped, the light turned off and the visual receptive field was mapped. The mapping was performed manually using a pantoscope (ISCO-Göttingen, PROJAR 1:3,5/150) that could provide output in the form of the rectangle of adjustable size while the subject was fixating the dot presented on the working panel. First, a big stimulus such as a long bar was projected on the panel and moved while monitoring neuronal response to coarsely determine the responsive zone of the visual field. The zone was recognized as responsive by associated significant increase in neuronal activity when the stimulus passed through it. Then, the output of the pantoscope was adjusted to be a small rectangular spot of less than 2 mm side length in order to precisely map the receptive field. After mapping the receptive field, the task started and, while the subject performed the task, the electrode was slowly advanced and the neuronal signal monitored. Initially, we advanced the electrode slowly the entire time of the recording. Since, the probability that the subject would stop to perform in the task was high, we later focused on the zone where the neurons activated in the task were likely to be found. Thus the electrode was advanced quickly to the zone below 2 mm. Then, it was stopped and advanced further only after a pause, usually 5 minutes, to allow the tissue dynamics to stabilize. Namely, it is believed that electrode due to friction moves the surrounding tissue in a certain extent in the direction itself moves. This causes slight compression, which in turn causes relaxation back-motion of the tissue. Amongst other, this underlies phenomena such as loosing the neuronal signal due to the motion of the recorded neuron away from the electrode tip or damaging the recorded neuron due to its motion and collision with the electrode tip despite holding the electrode in place. After the pause, the electrode was advanced slowly at a rate of approximately 1 μm per second with frequent stops. Upon isolating spikes of an individual neuron, the electrode was stopped and the neuronal signal monitored. If it
appeared that the signal is anyhow task-related the spike sorter was adjusted to isolate spikes of the neuron and the signal recording was initiated.

After noticing first signs of subject’s fatigue towards the end of the session such as breaking fixation or slowing arm movements the session was terminated upon a succession of few (usually four) consecutive successful trials. This termination of the session was accompanied by immediate positive reinforcement in a form of additional liquid reward (up to 50% of the acquired reward during the session) and pleasant social interaction with the subject. The purpose of this reinforcement was to promote that subject does not terminate the session on his own but continues to work until the session is externally terminated. This was critical for data collection since a significant number of trials were needed to successfully complete the session after finding an appropriate neuron.

We performed 341 penetrations, and for each we sought confirmation that the SC was acquired. If the subject did not want to visually fixate so that SC receptive field could not be confirmed, the recording results were not included in the data set. In majority of such cases, the electrode was retracted, as well as the guide tube and the session was continued without the recording. In some cases in the later stage of the study an obstacle in the tissue appeared to block the electrode penetration at approximately 1 mm below the dura at the penetration location that otherwise acquired the right lateral SC; in those cases recording was stopped as well. Finally, if the subject decided not to work in the task the recording was also terminated and this was the main reason that many penetrations have not resulted in data collection.

**Recording of the Perturbation Sound**

For the purpose of the testing for auditory response of perturbation-only neurons, the perturbation sound was recorded by a digital sound recorder (Olympus LS10) in a variety of different positions both in the room including the position where
the subject was situated during experiments and directly inside the attenuation box close to the sound source. Recorded sound files were evaluated and those that most closely resembled the original perturbation sound were selected for the testing of neurons. Importantly, these stimuli only resembled the original sound, however they were not identical to it and subjectively they were clearly different from the original.

**Attenuation of the Perturbation Sound**

To minimize the perturbation sound level, the valve box that controlled the inflow of the compressed air was placed in a custom-made attenuation enclosure. The enclosure (40 cm x 40 cm x 40 cm) was made of wood and inside cushioned with a dark sponge of 3 cm thickness with an increased active surface by fabricated knolls and troughs. Additional cushion made of 5 cm thick yellow sponge with fabricated holes was loosely wrapped around the valve box forming a thick, layered, irregular cushion and the box was placed in the center of the attenuation enclosure.

**Force Measurement**

Initially, a force sensor based on FSR technology was used (FSR151, IEE), which was suitable for the contact onset detection due to the very quick response. However, this sensor tended to saturate upon the subject exerting higher contact forces and thus was not suitable for accurate force measurement after the contact. Hence we replaced it with a one based on the strain gauge technology (KD40S, 100N, ME-Meßsysteme; sensor amplifier: GSV-2ASD, ME-Meßsysteme), which provided accurate force measurement. The output of the sensor system was additionally amplified (Tektronix AM502 differential amplifier) and directed to the A/D input of the Pentium 1 computer.

To test the sensor response time, we compared the onset of its output signal with the arrival time of the hand to the working panel. Specifically, we placed a small commercial semiconductor laser opposite to the photo-detector so that the laser
beam was parallel to the panel and very close to the target button. When the hand arrived at the panel it interrupted the beam providing a change in the photocell output signal. The signal from the photo-cell was amplified and used as a trigger for the oscilloscope (Tektronix AM502) and the signal from the force sensor was used as an input for the same oscilloscope. In this way, upon the hand interrupted the beam, the oscilloscope trace started and then upon the contact the trace change reflected the signal from the force sensor. The time delay between the hand arrival and the force sensor response was thus readable from the time division of the oscilloscope screen and it was less than 5 ms. Therefore, the response of the force sensor was delayed even less than 5 ms relative to the contact since the real contact occurs after the hand arrival.

Data Analysis

Shuffle Predictor Analysis

First, the arrays of individual trials had to be prepared for concatenation. Trials had different duration and, in addition, specific events occurred at different time points from trial to trial. This imposed a specific problem that will be described on the example of the cross-correlation between super arrays of neuronal activity and panel position. Let us for simplicity consider a two trials case: In the trial 1, contact occurs after 500 ms (all times are relative to the start of the trial), the panel starts to move at 650 ms, stops at 1500 ms, the neuron starts to fire at 800 ms, stops at 1200 ms, and the trial ends after 2000 ms. In the trial 2, all events are shifted 300 ms later. Then, after concatenation two super arrays do not match not due to a different response of the neuron but simply because the reference time point is shifted. For higher number of trials such phenomenon may be even more pronounced. Thus, before forming a super array one has to assure that all trials have the same duration before and after one reference point that is related to the event under examination.
To assure this, before concatenation arrays from individual trials were made equally long according to one standard length. As a reference time point for this standardization, the contact time point was used. Each array was cut before the contact time point to the length corresponding to the minimal time that elapsed from the beginning of the trial to the contact time (the minimum of all trials). After the contact time point each array was made to have length of the maximal time elapsed after the contact time point to the end of the trial; arrays were extended for the appropriate length except the trial that had the maximal length. Arrays corresponding to neuronal activity were extended with zero values, and arrays corresponding to force or panel position with baseline values that were present at the end of the trial. This processing did not affect data analysis since it was out of the time span of relevant events in the trial.

**Measure of Neuronal Activity**

Initially, as a measure of neuronal activity in data analysis, a spike density function was tested. First, we used a Gaussian function to substitute individual spikes and obtain continuous time function representing neuronal activity (Parzen 1962, Paulin 1992, Richmond et al., 1990). Specifically, at each time point when a spike occurred in a trial, one Gaussian function was placed. In this way, an array of Gaussian functions was obtained and they together summed to result in a so-called spike density function (SDF). This approach may provide an appropriate estimate of the firing rate however the result depends on the width of the used Gaussian function, which is set by the user and this step is arbitrary. In our case, the obtained SDF function appeared sparse despite consistent firing of neurons and underestimated actual firing rate. The result was improved if wider Gaussian functions were used. However, this caused SDF to span unreasonably in the time when the neuron actually did not started to fire yet. This effect is related to a general
theoretical problem about using Gaussian function to substitute individual spikes (e.g. Thompson et al., 1996). Namely, the function that substitutes spikes has a non-zero value also before the time when spike occurred.

Another approach was to use a function that resembles a post-synaptic potential caused by a spike (Thompson et al., 1996). At each time point when a spike occurred in a trial, one so called kernel function described by:

\[ R(t) = \left(1 - e^{-\frac{t}{t_d}}\right) \cdot e^{-\frac{t}{t_g}} \]

was placed (\(t\) – time, \(t_d\) – decay constant, \(t_g\) – growth constant). In this way, an array of such functions was obtained and they together summed to result in an SDF. Importantly, constants \(t_r\) and \(t_d\) correspond to the rising and decay time constant of the kernel function, respectively. By setting \(t_r\) to a small value, usually 1 ms and \(t_d\) to a larger value, usually 20 ms, a very quick raising and a slow decaying phase are achieved, respectively (e.g. Thompson et al., 1996). Such a method apparently results in a plausible estimate of a firing rate mostly for high firing rates. In the case of our neurons with firing rates below 50 spikes per second, this approach underestimated the firing rate, due to insufficient summation of the individual kernel functions. Namely, if two kernel functions are not close enough in time than the influence of the first one on the subsequent is low and the resulting function is simply a sequence of two individual kernel functions. We emphasize that the kernel approach has a general tendency to underestimate a firing rate due to the described phenomenon.

Finally, as a measure of neuronal activity we tested an instantaneous spike frequency. By definition, it directly provides a firing rate of a neuron. However, such a signal typically contains high value fluctuations due to variability of inter-spike intervals. It is usually assumed that this variability somehow presents neuronal noise.
However, each fluctuation of the neuronal signal may reflect a meaningful processing, and variability in the signal occurs due to different states of the brain in different time points. The complexity of such processing is difficult to describe in terms of controlled experimental parameters and the resulting variability in neuronal signal is often deemed as noise. On the other hand, only fluctuations that consistently occur across trials of the task may be considered as related to the task events. Here, we first computed an instantaneous spike frequency for individual trials. To obtain a measure of neuronal activity over ensemble of trials we calculated a mean of instantaneous spike frequency from individual trials. Then, a so-called running average running algorithm was applied on the obtained mean signal using a digital filter (Hamming, 1989) implemented in MATLAB® for the purpose of signal smoothing that removed only extreme signal fluctuations. This resulted in a reliable estimate of the firing rate across all neurons and we adopted the smoothed instantaneous spike frequency (SMIF) as a measure of average neuronal activity.

Statistics

Before applying a statistical test on a specific set of a data, it was tested whether the data distribution is Gaussian by applying Lillie or Kolmogorov-Smirnov test. If the hypothesis that the data distribution is normal could not be rejected with the significance level 0.05, we applied a test from the T test family (Student T test for testing the difference of the mean from zero, or Two-sample Student T test for testing the difference of means between the two groups of data), otherwise ranskum testing was applied (Wilcoxon signed rank or Wilcoxon rank sum test, respectively). Similarly, for testing effect of factors in multiple groups of data, ANOVA was applied if the hypothesis that the data distribution is normal could not be rejected with the significance level 0.05, and Kruskal-Wallis (non-parametric analogous to one-way
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