

HOW MOVING VISUAL STIMULI MODULATE THE ACTIVITY OF THE SUBSTANTIA NIGRA PARS RETICULATA

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Abstract—The orientation of spatial attention via saccades is modulated by a pathway from the substantia nigra pars reticularis (SNr) to the superior colliculus, which enhances the ability to respond to novel stimuli. However, the algorithm whereby the SNr translates visual input to saccade-related information is still unknown. We recorded extracellular single-unit responses of 343 SNr cells to visual stimuli in anesthetized cats. Depending on the size, velocity and direction of the visual stimulus, SNr neurons responded by either increasing or decreasing their firing rate. Using artificial neuronal networks, visual SNr neurons could be classified into distinct groups. Some of the units showed a clear preference for one specific combination of direction and velocity (simple neurons), while other SNr neurons were sensitive to the direction (direction-tuned neurons) or the velocity (velocity-tuned neurons) of the movement. Furthermore, a subset of SNr neurons exhibited a narrow inhibitory/excitatory domain in the velocity/direction plane with an opposing surround (concentric neurons). According to our results, spatiotemporally represented visual information may determine the discharge pattern of the SNr. We suggest that the SNr utilizes spatiotemporal properties of the visual information to generate vector-based commands, which could modulate the activity of the superior colliculus and enhance or inhibit the reflexive initiation of complex and accurate saccades. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: visual response map, saccade, artificial neuronal network, cat.

Elementary sensorimotor reactions such as startle reactions, the orienting reflex or reflexive saccades are controlled by forebrain structures, including one of the main output structures of the basal ganglia, the substantia nigra (SN) (Pollack, 2001; Afifi, 2003). Nigrotectal connections may serve as an important route for forebrain control over elementary sensorimotor reactions of the midbrain (Graybiel, 1978; Behan et al., 1987). The role of the primate SN in visually-guided saccades was extensively studied by Hikosaka and Wurtz (1983). They described the activity of substantia nigra pars reticularis (SNr) in behaving mon-

keys, reporting that these units decreased firing in response to stationary visual stimuli used as saccade targets. Their work established the saccade-initiation theory (Wurtz and Hikosaka, 1986), which assumes that suspension of tonic inhibitory GABAergic input by the SNr to the superior colliculus (SC) is a permissive step toward a subsequent saccadic eye movement. Their findings, with respect to response properties of SNr cells, were later extended by the observation of increased activity of some SNr cells in response to visual sensory stimulation (Magariños-Ascone et al., 1994; Handel and Glimcher, 1999) and modulation of SNr responses by moving visual stimuli (Schwarz et al., 1984; Nagy et al., 2005a,b, 2006). The latter is not surprising, since movement is an important natural behavioral stimulus, and motion-dependent forecasting is needed to accurately track and target moving stimuli.

The present study examines the nature of the encoding algorithm by which the SNr maintains its diversified function, by testing the relationship between the response characteristics of single SNr cells and the physical properties of moving stimuli. We hypothesize that the SNr may supply specific information about moving stimuli to the SC, which promotes the correct guidance of sight. Specifically, we wish to know whether these neurons have the ability to encode specific features of stimulus movement in their action potential trains, information which may help to orient attention toward the forecasted position of the stimulus. We also tested whether excitatory and/or inhibitory responses are stimulus-dependent, and whether they are present in separate neuronal populations. To answer these questions, we recorded and analyzed the responses of a large number of visually-active SNr neurons in anesthetized cats. Based on to the visual response characteristics of these neurons, we offer an expanded hypothesis concerning the role of the SNr in the control of saccade initiation and accompanying visuomotor processes.

EXPERIMENTAL PROCEDURES

Animal preparation and surgery

The experiments were carried out on 10 adult cats of both sexes, weighing from 2.5 to 4.0 kg. All procedures were carried out to minimize the number and the suffering of the animals. They followed the European Communities Council Directive of November 24, 1986 (86/609 ECC) and the National Institutes of Health Guidelines for the Care and Use of Animals for Experimental Procedures. The experimental protocol had been accepted by the Ethical Committee for Animal Research at the Albert Szent-Györgyi Medical and Pharmaceutical Center of the University of Szeged.

Anesthesia was initiated with ketamine hydrochloride (Calypsol, 30 mg kg⁻¹, i.m., Richter, Budapest, Hungary). After cannulation of the femoral vein and the trachea, the animals were placed

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Abbreviations: AEV, anterior ectosylvian visual area; DAergic, dopaminergic; KS, Kolmogorov–Smirnov; SC, superior colliculus; SN, substantia nigra; SNr, substantia nigra pars reticularis.

in a stereotaxic head holder. The wound edges and pressure points were treated generously with procaine hydrochloride (Procain, 1%, TEVA, Debrecen, Hungary). Anesthesia was continued with halothane (Narcotan, 1.6% during surgery and 1.0% during recordings, Zentiva, Praha, Czech Republic; the minimum alveolar concentration (MAC) level of halothane being held at 1 and 0.5, respectively), and was maintained for 3 to 5 days. The depth of anesthesia was monitored by regular inspection of the pupil size on the non-treated side, and the examination of electrocorticogram and electrocardiogram recordings. The animals were then immobilized with gallamine triethiodide (Flaxedyl, 20 mg kg⁻¹, i.v., Sigma, St. Louis, MO, USA). During the experiment, a solution containing gallamine (8 mg (kg h)⁻¹), glucose (Glucosum 40%, 10 mg (kg h)⁻¹, Pannonpharma, Pécs, Hungary) and dextran (Rheomacrodex 10%, 50 mg (kg h)⁻¹, Baxter, Plating, Germany) in Ringer's solution (B. Braun, Melsungen, Germany) was infused continuously at a rate of 3 ml (kg h)⁻¹. Atropine (atropinum sulfuricum, 0.1%, 0.2 ml, s.c., Egis, Budapest, Hungary) and ceftriaxone (Rocephin, 40 mg (kg day)⁻¹ i.m., Roche, Budapest, Hungary) were administered. The end-tidal CO₂ level and the rectal temperature were monitored continuously and kept constant at 3.8–4.2% and 37–38 °C, respectively.

Recording and stimulation

Electrophysiological single-cell recordings were performed extracellularly via parylene-insulated tungsten microelectrodes (AM System Inc., Carlsborg, WA, USA, 2 MΩ). Vertical penetrations were made into the SN between Horsley–Clark coordinates anterior 4–7 mm, lateral 4–6 mm, at a stereotaxic depth between 4 and 7 mm. Individual action potentials were selected with the help of a data acquisition system (SciWorks Datawave, Datawave Technologies, Berthoud, CO, USA). Putative GABAergic SNr neurons were selected via the duration of their action potentials and their spontaneous firing rate (Ungless et al., 2004).

At the end of each experiment, the animal was deeply anesthetized with pentobarbital (Euthanyl, 200 mg kg⁻¹, i.v., Bimeda-MTC, Cambridge, Canada) and transcardially perfused with 4% paraformaldehyde solution. Brains were removed, sliced into coronal sections of 50 μm, and stained with Neutral Red. The positions of the recorded neurons were localized on the basis of the tracks of the electrode penetrations, and their depths were related to the surface of the SN. The position of the uppermost neuron that could be distinguished as belonging to the SN based on firing rate and recording depths was used to define the dorsal nigral surface.

Visual receptive fields of the neurons were estimated subjectively by listening to the neuronal responses to the movements of a light spot generated by a handheld lamp. To quantitatively characterize the motion sensitivity of SNr neurons, we chose a very simple stimulus, which provides a high contrast ratio, and whose parameters are easy to quantify. Specifically, moving spots were projected onto a tangent screen (LCD projector, refresh rate 100 Hz; resolution 1280×720 pixels; contrast ratio: 1300:1; response time: 8 ms) centered on the area centralis and positioned at a distance of 57.3 cm from the eye of the animal. In agreement with previous reports, tested visual fields covered almost the whole visual field of the contralateral eye. Spots of two different diameters were used: 1° or 5°. A standard stimulus set of 80 stimulus parameter combinations was used to test the visual responsiveness of each neuron. Specifically, we moved each contrast spot in eight different directions within the projected space (0–315° in 45° increments, where 0° corresponded to straight up direction), with five different movement velocities (5, 20, 40, 80 and 160 °/s). For each recorded neuron, each stimulus combination was presented at least 10 times. For each trial control records were made for 1000 ms before presenting the stimulus (the prestimulus time), and then the moving stimulus was presented for an additional 1000 ms (the peristimulus time). The number and temporal distribution of the action potentials recorded during visual stimulations were stored for off-line

analysis (sampling rate: 20 kHz) and were visualized as peristimulus time histograms.

Analysis of the visual responsiveness of the SNr neurons

The data were analyzed with MATLAB[®] software (The Mathworks, Inc., Natick, MA, USA). The duration of the recording from each cell is proportional to the chance of losing it during the recording session (and also with the instability of the recording). Consequently, the high number of different stimulus parameters tested forced us to test only a limited number of repetitions for each different stimulus. The responses of the SNr neurons to visual stimuli in anesthetized cats were usually weaker than those of other visually responsive structures in the visual system. Moreover, the low number of cases made *t*-test comparisons between the pre- and peristimulus periods vulnerable to sudden bursts. For these reasons, this statistical procedure was poorly suited for this work. To obtain a parameter that reliably estimates the effect of the applied stimulus on the activity of a neuron based on a relatively small number of recorded trials, we tested the responses in overlapping narrow time windows with the help of the Kolmogorov–Smirnov (KS) test. While all of the prestimulus periods during the whole recording of a neuron contained spontaneous activity, we handled them as a common dataset for spontaneity. This dataset was tested for stability and homogeneity. Those recordings in which more than 5% of trials exceeded the ±1 SD range of mean spontaneous activity were considered to have inhomogeneous spontaneous activity (bursting, fluctuations, etc.), or to be a result of unacceptable isolation during recording, and were excluded from the analysis. With the help of this strict rule, we were able to minimize false detection of response fluctuations. Two hundred sequences of 200 ms were randomly selected from the dataset, and the firing rates of each 200-ms-long segment were calculated (Fig. 1A). In a second step, the firing rates for each stimulus combination were estimated separately. The firing rate of each 100-ms-long sequence (with 50% overlap) within the peristimulus period was compared with the previously created dataset (which represented the spontaneous activity) by using the KS test (Fig. 1B–D). We defined those 100 ms-long sequences of the peristimulus time as responses for which the KS test demonstrated a significant difference from the spontaneous activity. These “significant” segments are marked with colored bars in Fig. 2, which denotes the responses of an SNr neuron to various visual stimuli. To quantify the strength of a response, we summed the absolute values of the net firing rates (the difference of the stimulated firing rate and the spontaneous firing rate) during the significant sequences of the peristimulus intervals. We tested our estimation method on numerous negative control recordings (on recordings without stimulation), and also on previous well-characterized neuronal data. The KS test, combined with the calculation of net firing rates, proved to be an effective tool for estimation of the strength of a response, because it eliminates the disturbances caused by non-consequent burst-like activity among trials, and also gives a reliable quantification of the strength.

To visualize the stimulus preference of each neuron, two-dimensional color-coded maps were constructed, where the axes represented the independent stimulus parameters, and the color scale denoted the responses to the different parameter combinations.

Classification of response properties

Based on visual inspection of the response characteristics, we classified the SNr neurons into 10 different tuning types (for details concerning the preference types, see the Results section). To have an objective, reproducible method with which to sort the response maps into hypothesized classes, we used an artificial neural network containing 40 joints as an input layer and 10 joints as an output layer (representing the 10 different hypothesized

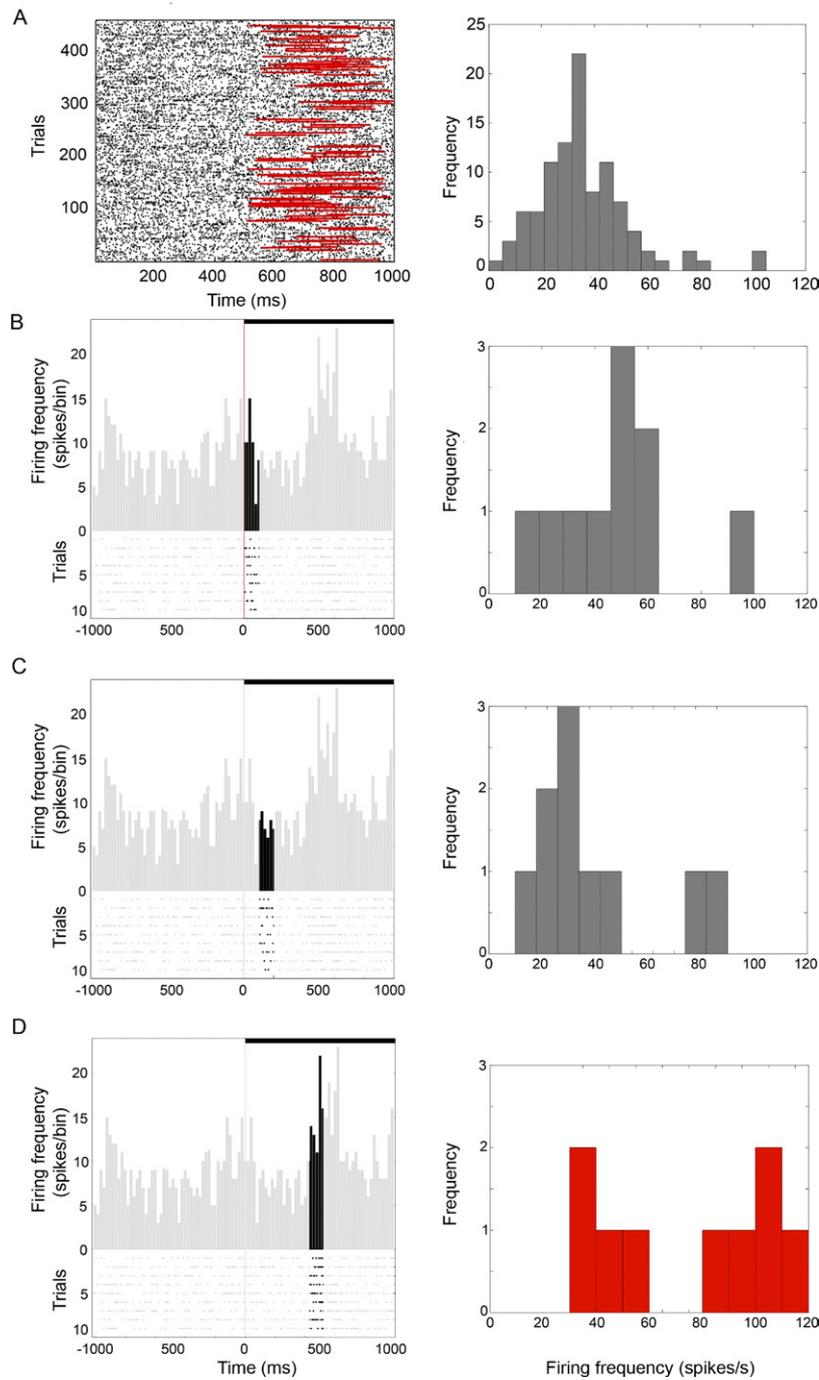


Fig. 1. Analysis of the visual responsiveness of SNr neurons. (A) The left panel depicts the raster plot of the spontaneous activity of a SNr neuron across 480 recorded trials, each 1000 ms long. The 200 randomly-selected, 200-ms-long segments which served as a representative dataset for the spontaneous firing rate of the investigated cell are indicated by red lines. The distribution function of this dataset can be seen in the right panel, where the abscissa defines the firing frequency ranges, and the ordinate the frequencies each range. (B, C) Two 100-ms-long segments of the neuronal activity during visual stimulation. The left panel shows the peristimulus time-histogram (PSTH) of a neuron. Each PSTH includes a prestimulus and a peristimulus period; the time scales are presented on the abscissas (ms). The duration of visual stimulation is denoted by the thick horizontal black line above the PSTHs, and ordinates demonstrate the cumulated spike counts in each 25-ms-wide bin. Below the PSTHs, the raster plots of the activities of the 10 trials are delineated. The unshaded part denotes the 100-ms-long segment of the response from which the distribution function in the right panel is calculated. The KS test indicates that these distributions in parts b and c are not significantly different from the distribution of spontaneous activity (part a, right panel). (D) A part of the PSTH which contains a visually-induced neuronal firing frequency increase; in this segment, the distribution of the firing rates differs significantly from that of the spontaneous activity. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

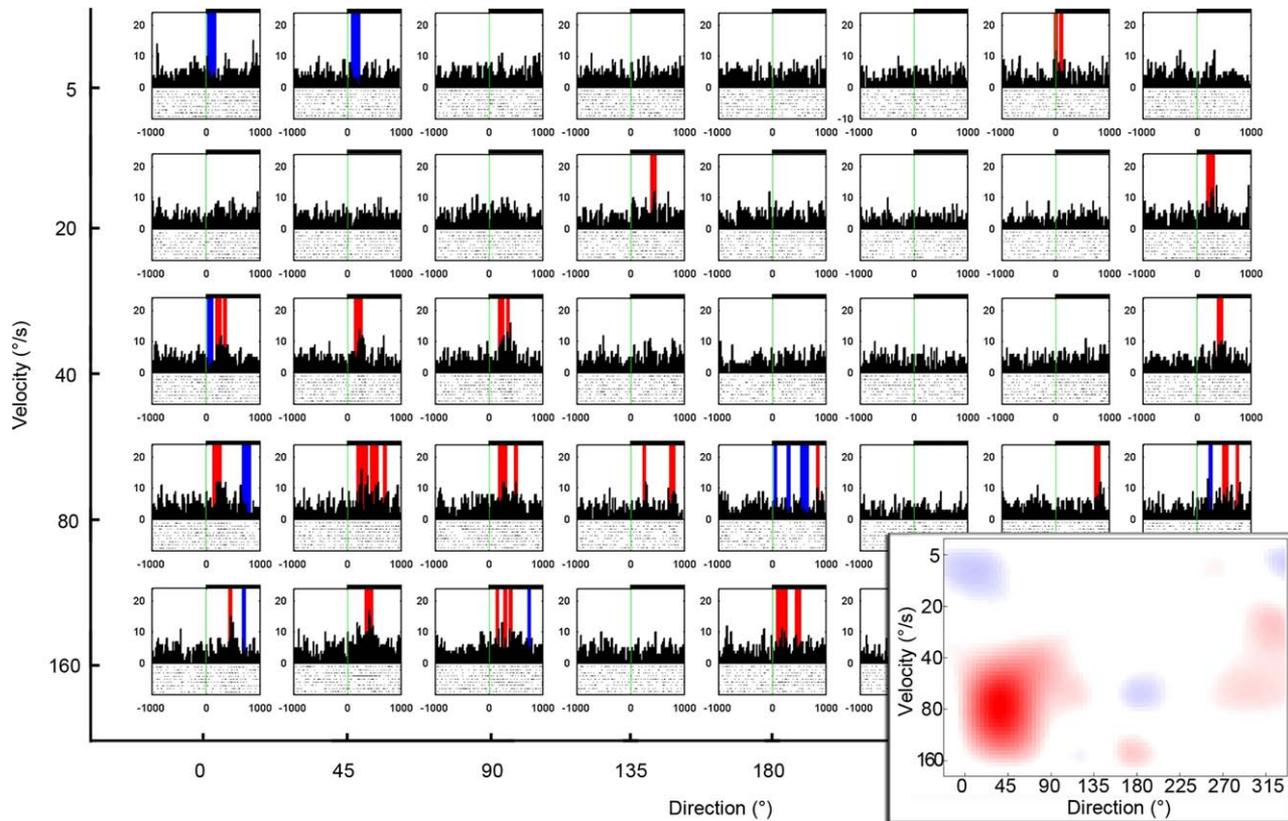


Fig. 2. Visual responses of a SNr neuron. The 5×8 peristimulus time-histograms (PSTHs) represent the responses of a SNr GABAergic neuron during the corresponding stimuli, with the same conventions as in Fig. 1. A significant increase in firing rate in a specific period in response to a given stimulus condition is indicated by a red bar, and a significant decrease by a blue bar. The direction and velocity of the movements of the applied stimuli are marked on the common abscissa and ordinate, respectively. In the lower left corner the corresponding response map can be seen as an inset. The axes denote the same direction and velocity values as the major figure's main axes. Inhibition and excitation are marked with proportionally dark blue and red colors, respectively.

classes), together with three hidden layers, each with 400 neurons. This network was automatically generated by a standard built-in function of the MATLAB software. The number of neurons within the different layers was adjusted empirically, to achieve the fastest, learning performance. The output layer was adjusted to have a log-sigmoid transfer function, and thus the response value of each output component ranged between 0 and 1. The system was embedded with resilient back-propagation as a self-learning algorithm. With this method, a properly trained network can correctly recognize inputs it has not seen before. (Because of the high number of possible variations within each group, a hypothetical definition of the classes is unavoidable; more objective unsupervised learning algorithms, such as the self-organizing map, cannot be used for this classification task.) This generalization ability was used to classify the response characteristics. The resilient back-propagation technique is widely used for automated, objective classification tasks due to its high internal stability (Serpen and Corra, 2002; Grip et al., 2003; review: Lotte et al., 2007). Above a certain complexity, neural networks of this kind show a rather stable performance (Palaniappan, 2006). Our choice concerning the number of neurons and hidden layers resulted in effective recognition performance, with acceptable computational costs.

To train the network, we generated artificial response characteristics (40–100 of each tuning type) and presented them to the network over several hundred epochs until the recognition error limit criterion (mean squared error, $<10^{-5}$) was met. After the training session, the real data for each recorded SNr neuron

were presented, and the network provided 10 values representing the probability of fit into the 10 different types of tuning characteristics. The schematic buildup of this artificial neural network is outlined in Fig. 3. For each automated classification result, we calculated the confidence value as:

$$V_c = 1 - \frac{P_2}{P_1},$$

where V_c is the calculated confidence value, while P_1 and P_2 denote the probability of the first and second most probable class type, respectively. We accepted a result as well-classified if the probability of the primary class was >0.5 , and its confidence value was >0.5 . If a response map did not meet this characterization criterion, then it was visually inspected. If the subjective categorization was in agreement with the automated result, the neuron was included within the analysis; otherwise we categorized it as “unclassified.”

RESULTS

Visual responsivity of the SNr neurons

The visual responses of 312 well-categorized SNr neurons, with monophasic spikes with waveform lengths of <1 ms indicating they were likely to be GABAergic (Grace and

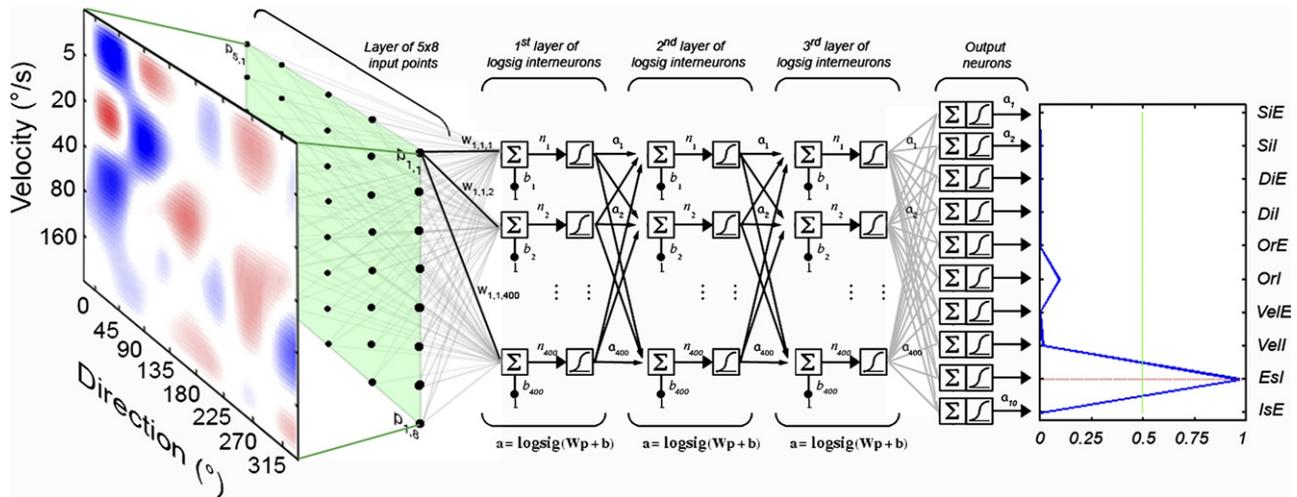


Fig. 3. Scheme of the artificial neural network used for response profile classification. The figure demonstrates the input dataset containing the response map of a recorded neuron, the layers of the artificial neural network, and the response characteristic profile produced by the network. The color coding and the conventions are the same as in Fig. 4. For a detailed explanation of the tuning of artificial neural layers, see the text.

Bunney, 1983; Ungless et al., 2004), were analyzed. The spontaneous activity of these neurons was high, with a mean of 27 spikes/s ($SD \pm 13$ spikes/s, range: 17–76 spikes/s). The 31 neurons in the unclassified group showed rather stochastic response maps, without any regular pattern. Interestingly many of these neurons had fluctuating responses, containing both excitatory and inhibitory segments. These single units were excluded from the further analysis.

The visual receptive fields of the classified neurons resembled those from our previous findings, since they covered most of the contralateral visual hemifield including the area centralis (Nagy et al., 2005a). The majority of the visually active neurons responded optimally to high velocities (>40 °/s), but poorly to stationary visual stimulation. No particular movement directions were preferred by a majority of the SNr neurons across the population. The spontaneous neuronal activity and velocity preference displayed a weak negative correlation; single units with higher spontaneous firing rate preferred lower stimulus velocities ($R = -0.12$, $P = 0.025$). We found no such correlation concerning the direction sensitivity.

The stimulus conditions which evoked the greatest change in neuronal activity were regarded as preferred conditions for the cells. There were 132 (42%) cells with dominant excitatory responses (an increase in their firing rate during stimulation). Their average firing rate in response to the preferred conditions was 34 spikes/s ($SD \pm 17$ spikes/s, range: 19 to 84 spikes/s), a 21% average increase relative to the spontaneous activity. For the 180 (58%) neurons with dominant inhibitory responses (a decrease in their firing rate during stimulation), the mean firing rate in response to the preferred conditions was 21 spikes/s ($SD \pm 11$ spikes/s, range: five to 68 spikes/s), 18% less than the mean spontaneous activity. Comparison of the spontaneous activity of the cells with the visual responses observed under the preferred conditions revealed significant differences for both the excitatory ($P < 0.01$) and

the inhibitory ($P < 0.01$) SNr neurons (Wilcoxon rank-sum test).

Velocity and direction tuning of the SNr visual responses

The major finding of this study is that a large proportion of the visual SNr neurons were not exclusively inhibitory or excitatory in nature, but could display either excitatory (increased activity) or inhibitory (decreased activity) visual responses, depending on the stimulus parameters. These SNr cells were uniform in the sense that they were able to respond with a definite increase in activity, but only under certain stimulus conditions (Fig. 2). These response characteristics were utilized to generate a map for each cell, and from these maps the neurons were classified into five categories (Figs. 4 and 5) with the help of an artificial neural network (see Experimental Procedures).

A majority of the neurons (61%, $n = 190$), referred to as simple cells, exhibited a simple stimulus preference, displaying significant changes in activity in response to well-defined stimulus combinations. These cells revealed a roughly circular region with narrow directional and velocity tuning in its best response zone (Fig. 4A, B), although minor regions with weaker responses were present. Eighty-one (26%) of these simple neurons responded predominantly with excitation (increased activity), while 109 (35%) underwent a definite inhibition (i.e. a decreased activity during visual stimulation; Fig. 5).

Fifty-seven (18%) of the recorded SNr cells, referred to as direction-sensitive neurons, responded to stimuli moving in a particular direction (Fig. 4C, D). In this case, the region of best response displayed a linear organization aligned along a specific direction. Seventeen (5.5%) of these cells exhibited an increase in activity in response to the preferred stimulus, and 40 cells (13%) showed a decrease (Fig. 5). No correlation was found between the directions evoking excitation and those evoking inhibition.

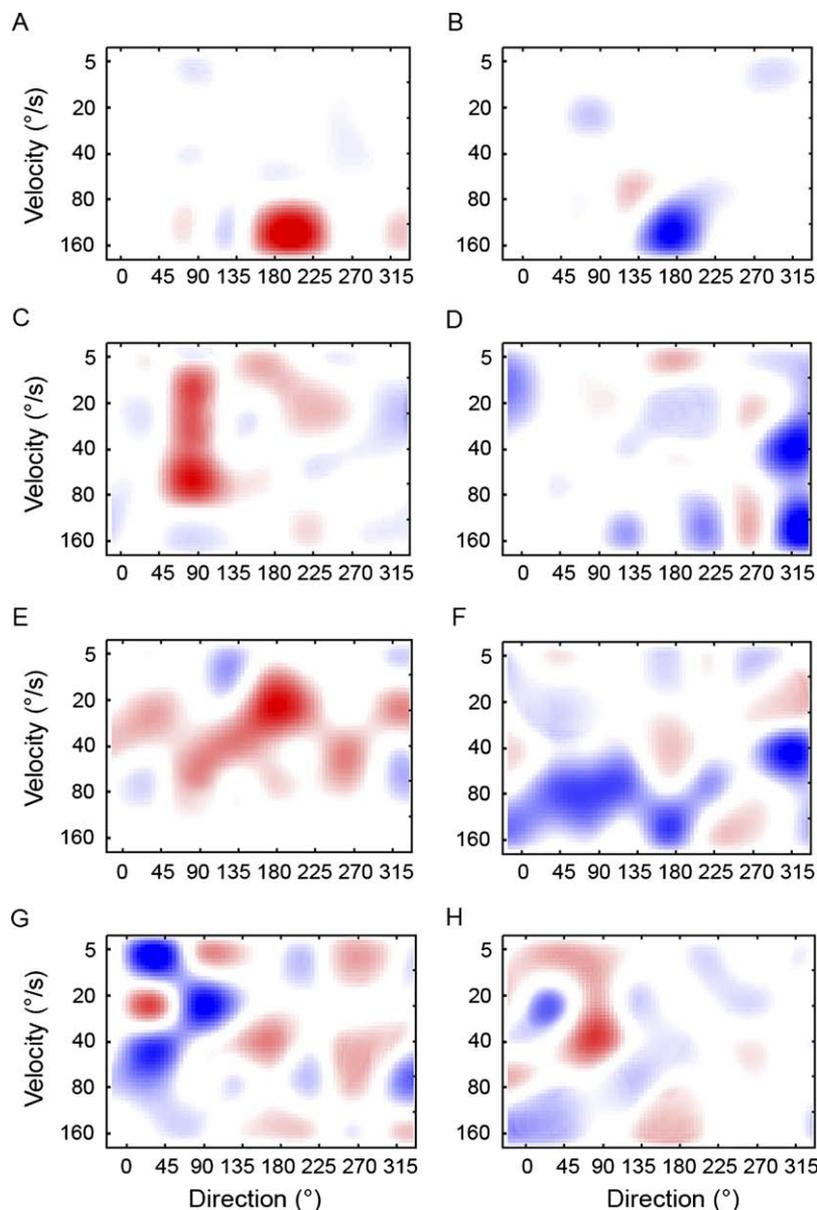


Fig. 4. Examples of visual response tuning maps. Each stimulus preference map shows the responsiveness of an SNr GABAergic cell with a specific type of preference. The abscissa denotes the eight different directions of stimulus movement, and the ordinate the different velocities. The strength of the response to each specific stimulus parameter combination is color-coded; inhibitory responses (firing frequency decreases during stimulation) are marked in blue, and excitatory responses (increase in firing rate) in red. The intensities of the colors are proportional to response strengths. For better visibility, the color codes of the preference maps are normalized and smoothed via a bicubic spline technique. The neuronal responses to the applied visual stimuli were classified into 4×2 distinct classes: (A, B) simple excitatory and inhibitory, (C, D) direction-sensitive excitatory and inhibitory, (E, F) velocity-sensitive excitatory and inhibitory and (G, H) concentric neurons. For further details concerning the preference classes, see the text.

Within the limits of the small sample, all directions occurred with the same frequency. No neurons were found in the SNr that preferred two opposing directions of movements.

Twenty (6.4%) of the recorded cells were observed to be velocity-sensitive neurons (Fig. 4E, F). These cells responded to stimuli moving at a certain velocity, irrespective of their direction, which mapped as a linear region of best response aligned with a specific velocity. Seven (2.2%) of the responses were excitatory and 13 (4.2%) inhibitory (Fig. 5). The distribution of the preferred veloci-

ties was not characteristic; all velocities occurred with similar frequencies.

Both the velocity- and direction-tuned classes displayed maps that had additional areas of weaker modulation. Often the response areas adjacent to the main response were of the opposite sign. This organization was even more striking in the remaining 45 neurons (14.4%), referred to as concentric neurons. These cells exhibited a specific pattern of complex response characteristics (Fig. 4G, H). Twenty-eight (8.9%) cells responded to a specific

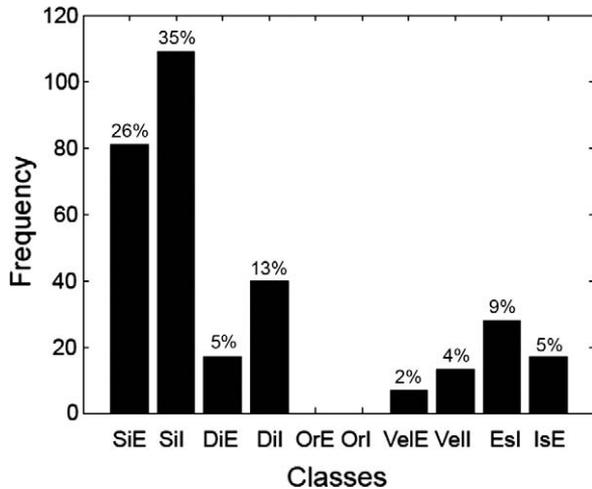


Fig. 5. Distribution of visual response characteristics of 312 neurons. The abscissa denotes the hypothesized 10 stimulus preference classes, while the ordinate shows the frequency of occurrence of each class. The percentage distributions are indicated above each bar. Abbreviations: SiE, simple excitatory; SiI, simple inhibitory; DiE, direction-sensitive excitatory; DiI, direction-sensitive inhibitory; OrE, orientation-sensitive excitatory; OrI, orientation-sensitive inhibitory; VelE, velocity-sensitive excitatory; VelI, velocity-sensitive inhibitory; EsI, concentric neuron—excitation surrounded by inhibition; IsE, concentric neuron—inhibition surrounded by excitation.

stimulus condition with an increase in activity, and to even slightly different neighboring conditions (both velocity and direction) with a definite decrease (Fig. 5). In other words, these neurons exhibited a simple response surrounded by an inhibitory domain in the velocity/direction plane. Conversely, 17 (5.5%) cells responded to a specific stimulus condition with a decrease in activity and to neighboring conditions with a definite increase (Fig. 5).

The neurons detailed above were distributed randomly in the SNr. No spatial clustering of the neuronal stimulus preference types was observed. Similarly, we did not find any correlation between the location of the cells and their velocity or direction preference.

Stimulus size modulation of the responses

To address the question of how the size of the stimulus affects the response characteristics of SNr neurons, light spots of 1° or 5° in diameter were used to elicit visual responses from 139 of the recorded neurons. Stimulation with the 5° light spot led to a mean decrease of 18% in the excitatory response and a mean increase of 40% in the inhibitory response. Thus, the excitatory response to the 1° spot was significantly stronger than that to the 5° spot (Mann–Whitney test, $P < 0.01$). In contrast, the inhibitory response was significantly stronger ($P < 0.01$) to the 5° spot than to the 1° spot. It was noteworthy that the overall responsivity remained the same for each cell, irrespective of the stimulus size; only the ratio of the excitatory and inhibitory responses changed. With the smaller spot as visual stimulus, 57% of the responses observed within the overall population were excitatory and 43% were inhibitory. The larger stimulus made the response character maps

more ambiguous, making the automated classification of these maps far less reliable than those of the small stimulus. Further investigations with more stimulus diameters are needed to decide whether the response class of each neuron is independent of stimulus size.

DISCUSSION

This study highlights the excitatory and inhibitory effects of moving visual stimuli on SNr neurons in anesthetized cats. This is a suitable model for investigating visual information-processing in the SN, since it lacks the numerous direct and indirect non-sensory influencing factors (e.g. reward prediction, unexpectedness and motor processes) present during behavioral paradigms (Sato and Hikosaka, 2002; Dommett et al., 2005; Hikosaka, 2007). However, anesthesia has a direct influence both on the spontaneous excitability and on the responsivity of the recorded neurons (Villeneuve and Casanova, 2003). In addition to the general depression, feedback information originating from the motor executor system is also absent from the investigated circuitry. Experiments carried out on awake animals usually report a lower proportion of excitatory type responses than those on anesthetized animals. This may be because the presence of higher spontaneous activity during awake conditions decreases the ability of excitatory SNr inputs to generate significant activity changes. By contrast, inhibitory inputs would have the opportunity for greater modulation of the SNr cell activity. The result would be a shift in balance toward inhibition under awake conditions. With the introduction of the sliding KS test into our analysis, the detection of presumed weakened responses was possible. However we have to emphasize the high computational cost of this analysis in contrast to the classic methods. A further disadvantage of this algorithm is the loss of the temporal distribution pattern, which may also carry important information. Below, we speculate on the role of the dualistic behavior of the SNr neurons, and propose a sequence of information coding with which the SNr may control the visuomotor functions of the SC.

Physiological properties of the recorded SNr neurons

On the basis of their neurotransmitters, most of the neurons of the SNr can be classified mainly into dopaminergic (DAergic) and GABAergic classes, although a few non-DAergic–non-GABAergic cells remain, whose transmitters have not yet been clarified. Ficalora and Mize (1989) demonstrated that the nigrotectal tract consists of the axons of GABAergic neurons. We selected presumed GABAergic cells from the neuronal population during recording by considering two properties: (1) these cells have narrow spike forms (~1 ms) in extracellular recordings, and can therefore be reliably differentiated from DAergic cells which produce broad spikes of over 2 ms that usually appear as spike doublets or triplets (Grace and Bunney, 1983; Ungless, 2004) and (2) GABAergic cells have been shown indirectly to be fast-firing type II cells, in contrast with the slow-firing type I DAergic cells (Guyenet and

Aghajanian, 1978). Based on electrode track reconstructions, the SNr neurons investigated in our study were located in the area of origin of the nigroretectal neurons (Beckstead et al., 1981; Jiang et al., 2003). Thus, their physiological properties and location indicate that the recorded neurons were likely GABAergic nigroretectal cells.

In general, most of the SNr neurons we studied exhibited sensitivity to moving visual stimulation. Earlier studies led to the categorization of GABAergic SNr neurons into two classes. Most of the neurons responded to visual stimuli with strong inhibition, while a smaller proportion increased their firing rate (Handel and Glimcher, 1999; Basso and Wurtz, 2002; Comoli et al., 2003). A main finding of the present study was that these neurons can be either excited or inhibited, depending on stimulus conditions. In our preliminary results larger stimuli elicit more intense inhibition in the SNr, while strongly decreasing excitatory responses, though the amount of total responses remained similar.

Based on our results, we propose a new classification of SNr neurons. In addition to the classes with narrowly tuned neurons displaying velocity and direction sensitivity, we have found neuronal classes which showed broad band tuning to one of these stimulus properties. These neurons may serve as stand-alone direction or velocity detectors, and may reflect a higher level of organization. Perhaps they receive multiple inputs from different simple cells and thus integrate their preference maps. This would also explain the existence of concentric preference maps, where the simple response conditions are surrounded by inverse responses. These neurons would disinhibit or block their target SC neurons, only if the movement of the stimulus precisely fit their preference. As a result, they would produce a vectorial saccade command for a specific direction and distance, consistent with previous evidence for complex direction and velocity tuning of SNr GABAergic neurons (Nagy et al., 2005a).

Similarities of stimulus preference between the SNr units and neurons of other visual structures

The source of the complex direction and velocity responses observed in SNr is currently unknown. Previous studies stimulating the anterior ectosylvian visual area (AEV) with simple geometric forms suggested that it also contains a high proportion of direction-selective units (Mucke et al., 1982; Benedek et al., 1988; Hicks et al., 1988). Direction-selective cells are also common in areas 17 (Hammond and Andrews, 1978), 18 (Rose and Blake-more, 1974), and 21b (Tardif et al., 2000) and in the posteromedial lateral suprasylvian area (PMLS) (Blake-more and Zumbroich, 1987), but are less common in areas 19 (Bergeron et al., 1998) and 21a (Wimborne and Henry, 1992; Dreher et al., 1993). Orientation selectivity, in which neurons are sensitive to movements along one axis, usually with inverse response to the opposing directions, is a characteristic feature of AEV units, but was not found in this investigation of the SNr. Inhibitory responses are a characteristic feature of cells in area 21b (Tardif et al., 2000) and also cells in the deep layers of

SC (Dreher and Hoffman, 1973). Detecting neurons with velocity and direction sensitivity (analogous to those discovered earlier in the SC and other structures along the extrageniculate–extrastriatal pathway (Benedek et al., 1996; Nagy et al., 2003; Waleszczyk et al., 2007)) suggests that the SNr receives strong modulation from this part of the visual system.

Visual afferents supplying the SNr

We did not detect any clustering or correlation between the position, direction or velocity preference of cells with different characters in the SNr. This supports the notion that the SNr is not retinotopically arranged or systematically mapped in any more complex fashion. Anatomical studies indicate two sets of neural circuits that may transmit visual information from the retina to the SN. A schematic representation of these anatomical connections is provided by Fig. 6. Visual information presumably reaches the SNr through the conventional corticostriatal route (Saint-Cyr et al., 1990; Norita et al., 1991). Visually active SNr neurons may also receive visual input through direct and indirect tectonigral pathways (Tokuno et al., 1994; Comoli et al., 2003). The SC also projects to the pedunculopontine nucleus (Redgrave et al., 1987) and the subthalamic nucleus (Tokuno et al., 1994; Coizet et al., 2009), and both of these nuclei make direct contact with neurons in the SN (Lokwan et al., 1999). It has been suggested that basal ganglia circuits including the subthalamic nucleus could be a source of visual information for the SN cells with excitatory responses (Jiang et al., 2003). Further, the SC sends strong visual efferents to the suprageniculate nucleus of the thalamus (Katoh et al., 1995), which in turn provides visual information to the caudate nucleus (Harting et al., 2001), thereby forming a tecto-thalamo-striatal route. Although the SN receives predominantly inhibitory inputs from the striatum, an excitatory striatonigral pathway (Rodríguez et al., 2000) may also transmit visual information to the nigral neurons.

Functional role of SNr GABAergic neurons

It is unclear whether the visually responsive SNr neurons take part in sensory information processing, or are instead components of the reverberating motor circuitry of the basal ganglia. The need for integration of sensory information and motor commands suggests the latter, but direct evidence can only be gained from awake, behaving animal experiments. The convergence of different sensory (Nagy et al., 2005b, 2006), oculomotor (Sato and Hikosaka, 2002; Hikosaka and Wurtz, 1983) and somatomotor (Schultz, 1986) information onto SNr neurons is well supported, but this does not mean that every sensory neuron has a role in motor functions. Based on previous studies, we conclude that saccade-related neurons that display visual activity are more likely to project to the SC than those without sensory activity (Hikosaka and Wurtz, 1983).

A shift of attention toward novel stimuli in the surrounding space requires an analysis of the movement parameters of the object by the saccade control system. Detection of novelty may be provided by DAergic SN cells (Martin

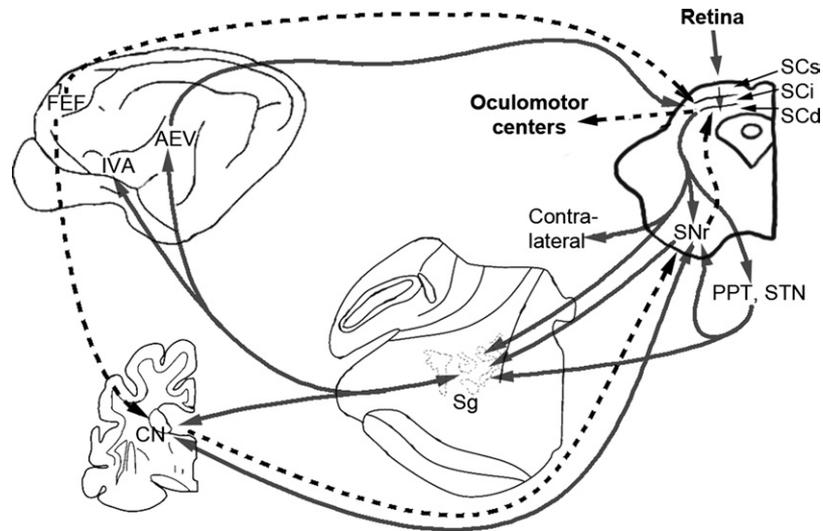


Fig. 6. Visual and oculomotor connections of the SNr. Connections within the ascending tecto-fugal visual system are marked with solid arrows, while the oculomotor circuitry is denoted with dashed arrows. Note that the ascending tecto-fugal pathway and the oculomotor pathway overlap each other. Abbreviations: SCs, SCi, SCd, superior colliculus superficial, intermediate and deep layers respectively; PPT, pedunculo-pontine-tegmental nucleus; STN, subthalamic nucleus; Sg, suprageniculate nucleus; CN, caudate nucleus; FEF, frontal eye field; IVA, insular visual area.

and Waszczak, 1994, 1996; Rice et al., 1997; Radnikow and Misgeld, 1998; Comoli et al., 2003; Dommett et al., 2005; Redgrave and Gurney, 2006). Thus, pars compacta cells may directly or indirectly confer the ability to appropriately modulate saccadic movements onto SNr cells. The fact that we found an equally represented preference for every stimulus direction and velocity combination suggests that, despite the absence of a topographic organization in the SNr, some kind of functional organization may be present. Wurtz and colleagues (Wurtz and Hikosaka, 1986; Basso and Wurtz, 2002) postulated a higher-order topographic organization between the visual stimulus site and the center of the receptive fields. We suggest that each SNr neuron might be connected to corresponding SC cells in such a way that they can activate or block specific saccades (McIlwain, 1990). The SC, on the other hand, receives a retinotopic, highly-ordered input both from the retina (Graybiel, 1975; Ogawa and Takahashi, 1981; Beckstead and Frankfurter, 1983) and from the cortical visual areas (McIlwain, 1977; Berson and McIlwain, 1983; Berson, 1988; Harting et al., 1992). These two projections converge onto SC neurons, and furnish its retinotopic organization (McIlwain, 1990). The simple SNr cells recorded here may activate or inhibit specific saccades that would drive the eye toward a position where a moving stimulus is predicted to be when the saccade is carried out (Jiang et al., 2003). Moreover, Kaneda and colleagues (2008) recently showed that the ipsilateral nigroretinal projection also targets GABAergic interneurons in the SC. By modifying the balance between the effect exerted directly on the projection neurons of the SC and through this indirect connection, the SNr can adjust both the spatial and temporal aspects of the activity of SC motor neurons.

The functional relation between the SNr and SC in pursuit eye-movements and saccade generation is known, but the code behind the information transmission remains

controversial. Two current theories about the meaning of population activity in the SC (Van Gisbergen et al., 1987; Lee et al., 1988; Van Opstal and Van Gisbergen, 1989) suggest that the activity of each ensemble determines a vector of eye movement with a definite amplitude and direction. This means that the SC expresses an integrated signal that takes into account both the actual eye position (McIlwain, 1990), and the expected future position. These might be integrated within the SC, or possibly within an external structure that serves as an integrative center. The inhibitory nigroretinal neurons presumably help to sharpen the collicular response map by disinhibiting some tectal units when they decrease their own firing rate, while suppressing other tectal units when they increase their firing rate. The capacity of the SNr signal to modify the saccade direction was verified recently by Liu and Basso (2008). They found that electrical activation of the GABAergic SNr cells profoundly modified the amplitude and direction of saccades in behaving paradigms, and the stimulation influenced both cell types in the SC. Li et al. (2006) concluded that as a further step of integration, the mutual inhibition among the populations of SC neurons will shape the sum gross response profile of the SC, forming a dynamic balance between inhibition and excitation caused by the SNr. This output signal (a dynamic sum of the “mini-vectors” represented by the single units of the active population) is suitable for driving brainstem oculomotor centers (Anderson et al., 1998). The vectorial response profiles of both the buildup and burst cells of the SC suggest that they are driven by two different sources: the incoming feedback signal reflecting the actual eye position (or the difference between the site of current attention) and information on the position (or expected position) of the point of interest. The input information is integrated in both the SNr and the SC, in multiple consecutive steps.

CONCLUSION

To summarize, this paper provides further understanding of how visual information may modulate the activity of the SNr neurons. The spatiotemporally represented visual information may determine the sensorimotor integrative function of the SNr. We suggest that the SNr could control the activity of the SC through direct nigrotectal connections, and could enhance or inhibit the reflex initiation of saccades to moving targets.

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APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at [10.1016/j.neuroscience.2009.07.031](https://doi.org/10.1016/j.neuroscience.2009.07.031).