Neuronal correlates of attention and its disengagement in the superior colliculus of rat

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Abstract

Orienting attention to a new target requires prior disengagement of attention from the current focus. Previous studies indicate that the superior colliculus (SC) plays an important role in attention. However, recordings of responses of SC neurons during attentional disengagement have not yet been reported. Here, we analyzed rat SC neuronal activity during performance of an attention-shift task with and without disengagement. In this task, conditioned stimuli (CSs) (right and/or left light-flash or sound) were sequentially presented. To obtain an intracranial self-stimulation reward, rats were required to lick a spout when an infrequent conditioned stimulus appeared (reward trials). In the disengagement reward trials, configural stimuli consisting of an infrequent stimulus and frequent stimulus in the former trials were presented; in the non-disengagement reward trials, only an infrequent stimulus was presented. Of the 186 SC neurons responding to the CSs, 41 showed stronger responses to the CSs in the disengagement reward trials than in the non-disengagement reward trials (disengagement-related neurons). Furthermore, lick latencies in the disengagement reward trials were negatively correlated with response magnitudes to the CSs in half of the disengagement-related neurons. These disengagement-related neurons were located mainly in the deep layers of the SC. Another 70 SC neurons responded to the CSs in both disengagement and non-disengagement reward trials, suggesting that these neurons were involved in attention engagement. Our results suggest complementary mechanisms of attentional shift based on two subpopulations of neurons in the SC.

Key words: attention; disengagement; single-unit recording; superior colliculus; rat

Introduction

The superior colliculus (SC) constitutes part of the brain network involved in visual attention (Shipp, 2004; Krauzlis et al., 2013), and contributes to overt attention by controlling motor outputs (Sparks, 1999, 2002; Krauzlis, 2003) and target selection processes (McPeek and Keller, 2002, 2004; Nummela and Krauzlis, 2010). This structure also plays a crucial role in covert attention; inactivation of the primate SC impairs covert selection of signals for perceptual judgments (Lovejoy and Krauzlis, 2010). Further, microstimulation of the primate SC focuses attention without movement of eyes (Müller et al., 2005). Consistently, visuomotor neurons in the monkey SC were found active during covert shift of attention (Ignashchenkova et al., 2004). Non-invasive human imaging studies have also reported that the human SC is active during selective attention (Corbetta et al., 1991; Schneider and Kastner 2009). These studies indicate a crucial role of the SC in the orientation of attention.

Orienting attention to a new target requires three sequential mental operations: 1) disengagement of attention from its current focus; 2) moving attention to the new target; and 3) engagement of the new target (Posner et al., 1984; Posner and Petersen, 1990). Thus, the process of attentional disengagement is a primary initial step in orienting. A behavioral study reported that reaction times to make a saccade to a peripheral target are faster when a central fixation point goes off shortly before target presentation (gap trials) than when the central fixation stimulus stays on (overlap trials) (Saslow, 1967). This is because the subjects must disengage attention from the central target before shifting attention to the peripheral target in the overlap trials, and disengagement of attention takes time (Fischer and Breitmeyer, 1987). Despite the importance of the disengagement process, the neural mechanisms underlying disengagement processing are still poorly understood. To date, only four studies have focused on the neural mechanisms that are

related to visual disengagement from fixation. These clinicopathological and electroencehphalography (EEG) studies have suggested that the frontal eye fields (Rivaud et al., 1994) and parietal lobe (Posner et al., 1984; Csibra et al., 1997) might be involved in disengaging attention. Studying a patient with lesions in the right SC revealed that mean saccade latency to the contralateral peripheral target was longer in overlap trials than in gap trials (Pierrot-Deseilligny et al., 1991). This suggests that the SC is also involved in attentional disengagement. However, no previous neurophysiological studies have investigated neural mechanisms of attentional disengagement in the SC. In the present study, we analyzed rat SC neuronal activity during performance of an attention-shift task with and without disengagement. Here, we report that a population of SC neurons strongly responded to visual cues in trials requiring attentional disengagement.

Materials and Methods

Subjects

Eleven male Wistar rats, weighing 270–320 g at the time of surgery (12–16 weeks old; SLC, Hamamatsu, Japan), were used. The rats were individually housed in a room where temperature (24 ± 1°C) and light (07:00–19:00) were automatically controlled. Food and water were available ad *libitum*. Treatment of all rats was in strict compliance with the United States Public Health Service Policy on Human Care and Use of Laboratory Animals, National Institutes of Health Guide for the Care and Use of Laboratory Animals, and Guidelines for the Care and Use of Laboratory Animals at the University of Toyama. All experimental procedures were approved by our institutional committee for experimental animal ethics.

Surgery

Surgery was performed under aseptic conditions in two stages. First, a cranioplastic cap was attached to the skull as described in our previous studies (Uwano et al., 1995; Nishijo et al., 1998). This cap was used for the head restraint system for wakeful rats and was identical to that of Nishijo and Norgren (1990). The rat was anesthetized (sodium pentobarbital, 40 mg/kg; intraperitoneal, i.p.) and then mounted in a stereotaxic apparatus. The skull was exposed, and five small sterile stainless screws were threaded into holes in the skull to serve as anchors for cranioplastic acrylic. Two bipolar electrodes for intracranial self-stimulation (ICSS) were implanted in the peduncular part of the lateral hypothalamus (A, −3.36 from bregma; L, ± 2.0; V, 9.2), according to the atlas of Paxinos and Watson (2007). Then, the cranioplastic acrylic was built up on the skull and molded around the conical ends of two sets of stainless steel bars. During subsequent surgery or during the recording session, the double end of these artificial earbars served the same function as regular earbars and could be used in the unanesthetized animal without inducing pain. A short length of 27-gage stainless-steel tubing was embedded into the cranioplastic acrylic near the bregma to serve as a reference pin. After surgery, an antibiotic (gentamicin sulfate, Gentacin® injection, Schering-Plough, Osaka, Japan) was administered topically and systemically (2 mg; intramuscular, i.m.).

After recovery from surgery (5–7 d) and after training (7–10 d, see below), rats were again anesthetized (sodium pentobarbital, 40 mg/kg, i.p.) and mounted with the artificial earbars. A small hole (A, −8.0 to –5.0 from bregma; L, 0.0–2.0 right or left) was drilled through the cranioplastic acrylic and the underlying skull for chronic, repeated recordings. The exposed dura was excised, and the hole was covered with hydrocortisone ointment (Rinderon-VG® ointment, Shionogi Co., Ltd., Tokyo, Japan); alternatively, one or two drops of chloramphenicol

(Chloromycetin® succinate, Sankyo Co., Ltd., Tokyo, Japan) solution (0.1 g/mL) were dropped into the hole. The hole was covered with a sterile Teflon sheet and sealed with epoxy glue.

Training and task paradigms

Before surgery, the rats were acclimated by handling and were accustomed to being placed into a small, plastic restraining cage for brief periods. After recovery from the first stage of surgery, the threshold level for ICSS was determined, and any rat for which the threshold exceeded 300 μA was excluded. Then, the rat was trained to perform the attention-shift task with and without attentional disengagement.

During task training, the rat was placed in the restraining cage with its head fixed rigidly and painlessly in the stereotaxic device by the artificial ear bars. A midrange speaker, located 1 m in front of the rat, delivered the auditory stimuli, and each white light, 3 cm in front of each eye, delivered the visual stimuli (Fig. 1A). The attention-shift task included 5 sessions, and each session consisted of 36 trials including 12 reward trials (infrequent trials) and 24 nonreward trials (frequent trials). In each trial, a CS (light flash or sound) appeared for 1 s, followed by spout protrusion close to the mouth for 2 s. In the reward trials (but not nonreward trials), rats could obtain ICSS rewards if they licked the spout (Fig. 1B). A touch sensor detected individual spout licks.

The sequence of the CSs in each session are shown in Fig. 1C. In all sessions, two kinds of CSs (infrequent and frequent) were sequentially presented, and when rats detected infrequent CSs and licked the spout, rats could acquire ICSS reward (reward trials). Trial sequence was set pseudo-randomly by a computer in that at least 1 nonreward trial always preceded each reward trial. Thus, the task required shift of attention to infrequent CSs. In session 1, right light associated

with nonreward was sequentially presented (nonreward trials), and when the left light, an infrequent stimulus, appeared, the rat could acquire ICSS reward if it licked the spout (reward trials). In session 2, left light associated with nonreward was sequentially presented (nonreward trials), and when right light, an infrequent stimulus, appeared, the rat could acquire ICSS reward if it licked the spout (reward trials). In sessions 3 and 4, right or left light associated with nonreward was similarly sequentially presented (nonreward trials), and when both right and left lights were simultaneously presented, the rat could acquire ICSS reward if it licked the spout (reward trials). The CS in the reward trials included not only the infrequent stimuli but also frequent stimuli of the former nonreward trials. Therefore, in sessions 3 and 4, the rat must disengage attention from the frequent CSs to detect the infrequent stimuli. In session 5, a tone (frequent stimulus) was sequentially presented, and when both right and left lights (infrequent stimuli) were simultaneously presented, the rat could acquire reward if it licked the spout. Session 5 was used as a control session for sessions 3 and 4; the CSs in the reward trials in session 5 were same as those in sessions 3 and 4, although attentional disengagement from the CSs in the former trials was not required. Sequence of the sessions was run pseudo-randomly. The rats were trained to lick the spout only in the reward trials.

Electrophysiological procedures and data acquisition

After the rats had learned the conditioned licking task to discriminate the CSs in the reward and nonreward trials, SC neurons were recorded during performance of the task. Neuronal activity of an individual rat was usually recorded every other day. After being placed in the enclosure, the ointment was removed, and a glass-insulated tungsten microelectrode ($Z = 0.5–1.5$ MΩ at 1 kHz) was stereotaxically and vertically inserted into the SC in a stepwise fashion by a pulse

motor-driven manipulator (SM-20, Narishige, Tokyo, Japan). Extracellular neuronal activity was passed through a multi-channel differential amplifier with a preamplifier (PBX, Plexon Inc., Dallas, TX, USA), monitored on an oscilloscope, and recorded on a data recorder (RD-135T DAT DATA RECORDER, TEAC). Only neuronal activity with a signal-to-noise ratio >3:1 was recorded. The analog signals of amplified neuronal activity, triggers for CSs, ICSS reward, and spout licking were digitized at a 40-kHz sampling rate and stored on a computer via a multichannel acquisition processor system (MAP, Plexon). The digitized neuronal activities were isolated into single units by their waveform components using the Offline Sorter program (Plexon). Spike sorting was performed with the offline sorter program for cluster analysis (Offline Sorter). Each cluster was checked manually to ensure that the cluster boundaries were well separated and the waveform shapes were consistent with action potentials. For each isolated cluster, an autocorrelogram was constructed and only units with refractory periods >1.2 ms were used for further analyses. Finally, superimposed waveforms of the isolated units were drawn to check the consistency of the waveforms. These units were transferred to the NeuroExplorer program (Nex Technologies, MA, USA) for further analyses.

Analysis of the basic characteristics of the SC neurons

Since the rats had to adapt to new rules in the beginning of the sessions, initial trials in each session were discarded and only data after the third reward trial were analyzed. Both neuronal and behavioral data on each trial were counted from the peristimulus histograms in successive 50-ms bins for three periods: a pretrial control period (500 ms), a CS stimulation period (1,000 ms), and rewarding stimulation (reinforcement) period (2,000 ms). Significant excitatory or inhibitory responses to each CS were defined by a Wilcoxon signed rank (WSR) test ($P < 0.05$) of neuronal activity between the 500-ms pretrial control period and the 1,000-ms CS periods.

Neuronal response magnitude was defined as follows: the mean firing rate during the 1,000-ms CS period minus the mean firing rate during the 500-ms pretrial period. For each neuron, neuronal response magnitudes to all CSs were compared by the one-way analysis of variance (ANOVA) and Tukey post-hoc tests ($P < 0.05$). Based on response patterns to the CSs, we then classified SC responsive neurons (see **Results**).

To analyze latencies of neuronal responses, one peri-event histogram was constructed using all data of each neuron. Neuronal response latency was defined as the interval from the onset of stimulus presentation to the time at which neuronal firing rate exceeded the mean ± 2.0 SD of the baseline firing rate. Mean response latencies to the CSs were compared among SC neuronal types by one-way ANOVAs at a significance level of $P < 0.05$. The post hoc comparisons were performed using Tukey tests with a significance level of $P < 0.05$. All data are expressed as mean ± standard error of mean (SEM).

For some SC neurons (attentional disengagement-related neurons, see **Results**), not only response magnitudes, but also response latencies and durations were analyzed in each CS. For this purpose, peri-event histograms of individual CSs were constructed. Neuronal response duration was defined as the duration during which the neuronal firing rate exceeded the mean ± 2.0 SD of the baseline firing rate. Because these neurons showed no responses in the nonreward trials, we analyzed latencies and durations of neuronal responses to each stimulus only in the reward trials. Mean latencies and duration of responses to the CSs were similarly compared among the CSs by one-way repeated-measure ANOVAs at a significance level of $P < 0.05$.

Analysis of lick latencies and their correlation with neuronal activities

Lick latency was defined as the interval from spout protrusion to the moment when the rat licked the spout. Mean lick latencies in reward trials were compared among the 5 sessions by

one-way repeated-measure ANOVAs. The post-hoc comparisons were performed using the Bonferroni-correction method with a significance level of $P < 0.05$. Correlations between individual lick latencies in the reward trials and neuronal activity were analyzed using simple linear regression.

Histology of the recording sites

After the last recording session, rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and several small electrolytic lesions (20 μA for 20 s) were made stereotaxically around the recording sites with a glass-insulated tungsten microelectrode. Rats were then given an additional overdose of anesthetic and perfused transcardially with heparinized 0.9% saline followed by 10% buffered formalin. The brain was removed and cut into 50-μm frontal sections with a freezing microtome. Sections were Nissl-stained with cresyl violet. The sites of electrical lesions were carefully determined microscopically. The location of each recording site was then calculated by comparing the stereotaxic coordinates of the recording sites with those of the lesions. Positions of neurons were stereotaxically located on the tissue sections and plotted on the corresponding sections on the atlas of Paxinos and Watson (2007).

Based on the locations of SC neurons, the ratio of SC neurons in the superficial layers, the intermediate layers and the deep layers were calculated for each type of SC neuron. The ratio (percent) of each layer for each type of SC neuron was defined as follows: (the number of a given type of SC neuron x 100)/the number of SC neurons recorded in that layer. The ratio of SC neurons among the different SC layers was compared with a Chi-squared test ($P < 0.05$).

Results

Lick latencies

During the task, rats almost always licked the spout in the reward trials, while they seldom licked the spout in the nonreward trials. Therefore, lick latencies were analyzed in the reward trials. We analyzed the behavioral data recorded from 156 responsive SC neurons with lick latencies less than 300 ms. Figure 2 shows the mean lick latencies in the reward trials of the 5 sessions (R1 to R5). Statistical comparison indicated that there was a significant main effect of session [$F(4, 152) = 23.216$, $P < 0.001$] (one-way repeated-measures ANOVA). Post-hoc tests indicated that mean lick latencies in sessions 3 and 4 requiring disengagement (R3, R4) were significantly longer than those in the other sessions requiring no disengagement (R1, R2, and R3) (Bonferroni tests, $P < 0.001$). These results indicate that attentional disengagement processes in sessions 3 and 4 delayed lick latencies, consistent with the idea of Fischer and Breitmeyer (1987).

Classification of SC neurons

Over a period of 1 to 3 months for each rat, recordings were made from 611 neurons located in and around the SC during the attention-shift task. Of these neurons, 583 were located in the SC. Table 1 summarizes the response patterns of these 583 neurons. One hundred and eighty-six (31.9%) neurons responded to the CSs. These 186 responsive neurons were classified into four types: disengagement-related neurons (22.0%, 41/186), reward and attention shift-related neurons (37.6%, 70/186), visually-responsive neurons (33.9%, 63/186), and inhibitory-responsive neurons (6.5%, 12/186).

Attention disengagement-related neurons

Disengagement-related neurons were defined as neurons that showed excitatory responses to the infrequent CSs requiring attentional disengagement (CSs in the reward trials in sessions 3

and 4) contralateral to the recording sites (WSR test, $P < 0.05$); they also included neurons with significantly higher response magnitudes to these CSs than to other CSs associated with and without reward (Tukey test after one-way ANOVA, $P < 0.05$). A typical example of this type of neuron is shown in Fig. 3A. The neuron responded to all infrequent CSs associated with reward in sessions 1 to 5 (Fig. 3A, R1 to R5) (WSR test, $P < 0.05$), but not to frequent CSs associated with nonreward (Fig. 3A, N1 to N5) (WSR test, $P > 0.05$). Comparisons of the response magnitudes to the CSs are shown in Fig. 3B. The response magnitudes to the infrequent CSs contralateral to the recording site requiring attention disengagement in session 3 (R3) were significantly stronger than those to the other CSs with and without disengagement (Tukey test after one-way ANOVA, $P < 0.01$). We found that this SC neuron was recorded from the right CS, and the right light was the frequent CS in session 3. To detect the left light associated with reward, the rat must disengage attention from the right light in session 3. On the other hand, the rat had to detect the left light in session 1 and the left and right lights in session 5, which required no disengagement. The above results indicate that the response magnitude to the CS requiring disengagement in session 3 was significantly larger than to the same CSs requiring no disengagement in sessions 1 and 5.

Figure 4 shows mean response magnitudes (a), mean response latencies (b) and the mean response durations (c) of the disengagement-related neurons recorded from the left SC (A), right SC (B), and both sides of the SC (C). In the left SC neurons ($n = 22$) (A), the mean response magnitude was significantly stronger (a), the mean response latency was significantly shorter (b) and the mean response duration was significantly longer in session 3 requiring disengagement than those in other sessions (Bonferroni test after one-way repeated-measures ANOVA; $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively). In the right SC neurons ($n = 19$) (B), the mean response magnitude was significantly stronger (a), the mean response latency was significantly shorter (b) and the mean response duration was significantly longer in session 4 requiring disengagement

than those in other sessions (Bonferroni test after one-way repeated-measures ANOVA; $P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively). In SC neurons bilaterally (n = 41) (C), the mean response magnitude was significantly stronger (a), the mean response latency was significantly shorter (b) and the mean response duration was significantly longer in sessions 3 and 4 requiring disengagement than those in other sessions (Bonferroni test after one-way repeated-measures ANOVA; $P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). These results indicate that the disengagement-related neurons responded stronger and faster to the CSs in the contralateral visual field requiring attentional disengagement.

The above results suggest that activity of this type neurons might correlate with lick behaviors in the sessions requiring disengagement. Figure 5 shows the relationships between neuronal response magnitudes in R3 shown in Fig. 3 and lick latencies in individual trials. Statistical analysis by simple linear regression indicated that there was a significant negative correlation between response magnitudes and lick latencies [($F(1,7) = 9.11$, $P = 0.019$; $r = –0.75$)]. Thus, stronger neuronal responses were accompanied by the shorter licking latencies. Of the 41 disengagement-related neurons, 17 (17/41, 41.5%) [left SC, 9/22 (40.9%); right SC, 8/19 (42.1%)] showed similar significant negative correlations between neuronal response magnitudes in session 3 or 4 with disengagement and lick latencies (r ranging from –0.68 to –0.93; $P < 0.05$, simple linear regression). Furthermore, another 5 neurons tended to show similar significant negative correlations (r ranged from –0.60 to –0.78; $P < 0.1$, simple linear regression). These results indicate that the attention disengagement-related neurons guide behaviors in the trials requiring attentional disengagement.

Reward and attention shift-related neurons

Reward and attention shift-related neurons were defined as neurons that showed excitatory

responses to the infrequent CSs in reward trials contralateral to the recording sites (e.g., CSs in R1, R2, and R3 for the SC neurons in the right SC) (WSR test, $P < 0.05$), regardless of attentional disengagement and if response magnitudes to these CSs were larger than those to the frequent CSs without reward (Tukey test after one-way ANOVA, $P < 0.05$). In 46 of the 70 rewards and attention shift-related neurons, responses to the infrequent CSs contralateral to the recording sites were larger than those to the infrequent CSs ipsilateral to the recording sites (Tukey test after one-way ANOVA, $P < 0.05$). A typical example of this type of neuron recorded from the right SC is shown in Fig. 6. This neuron showed excitatory responses to all infrequent CSs including those contralateral to the recording site (CSs in R1, R3, and R5) that were associated with reward (WSR test, $P < 0.01$), and not to the frequent CSs associated with nonreward (WSR test, $P > 0.05$) (A). Comparison of the response magnitudes to the CSs are shown in Fig. 6B. The response magnitudes to the infrequent CSs contralateral to the recording site (CSs in R1, R3, and R5) were significantly stronger than that to the CSs associated with nonreward (CSs) (Tukey test, $P < 0.001$), and also larger than those to the infrequent CSs ipsilateral to the recording site (CSs in R2 and R4) (Tukey test, $P < 0.05$). We found that the response magnitudes to the configural CSs in session 4, in which left light was the frequent stimuli (i.e., non-target), was significantly smaller than those to the same configural CSs in session 3, in which left light was the infrequent stimuli (i.e., target). These results indicate that this neuron responded stronger to the same contralateral CS (left light) when the rat attended to it than when the rat did not attend to it, suggesting that activity of this neuron reflects visual attention.

Visually-responsive neurons

Visually-responsive neurons were defined as neurons that showed excitatory responses (WSR test, $P < 0.05$) to the visual CSs (light flash) contralateral to the recording sites regardless of

reward association. A typical example of this type of neuron recorded from the left SC is shown in Fig. 7. The neuron responded to the CSs that included the right light (R2-5, N1, and N3) regardless of reward association (WSR test, $P < 0.001$) (A). Comparison of the response magnitudes to the CSs are shown in Fig. 7B. The response magnitudes to the CSs that included the right light were significantly stronger compared to those to the other CSs (Tukey test after one-way ANOVA, $P < 0.001$).

Inhibitory-responsive neurons

Inhibitory-responsive neurons were defined as neurons that showed inhibitory responses to some of the CSs (WSR test, $P < 0.05$). This type of SC neuron sometimes showed transient excitatory responses to those CSs in short latencies. Of the 12 inhibitory-responsive neurons, 4 neurons showed inhibitory responses to the CSs associated with reward if the CSs included the light in the contralateral visual field. These neurons showed responses similar to reward and attention shift-related neurons. A typical example of this type neuron recorded from the right SC is shown in Fig. 8. This neuron showed inhibitory responses to the infrequent CSs contralateral to the recording site that were associated with reward regardless of attention disengagement (WSR test, $P < 0.001$), and not to the frequent CSs associated with nonreward (WSR test, $P > 0.05$) (A). Comparison of the response magnitudes to the CSs are shown in Fig. 8B. The absolute values of the response magnitudes to the infrequent CSs contralateral to the recording site were significantly stronger than responses to the other CSs (Tukey test after one-way ANOVA, $P < 0.001$). The remaining 8 inhibitory-responsive neurons showed inhibitory responses to the contralateral light regardless of reward.

Response latencies and recording sites of SC neurons

The mean response latency of the visually-responsive neurons was short (26.5 ± 0.7 ms). However, attention disengagement-related neurons (157.2 ± 21.9 ms), reward and attention shift-related neurons (130.4 ± 13.3 ms), and inhibitory-responsive neurons (123.0 ± 20.2 ms) showed significantly longer mean response latencies (Tukey test after one-way AVOVA, $P < 0.001$).

The recording sites of all SC neurons are shown in Fig. 9. Of the 583 SC neurons recorded, 212 were located in the superficial layers (Zo, SuG, Op), 181 in the intermediate layers (InG, InWh), and 190 in the deep layers (DpG, DpWh). The ratios of neurons in each layer of each neuronal type are shown in Fig. 10. For the disengagement related-neurons, the ratios of the deep layers were significantly higher than those of the intermediate and the superficial layers (Chi-squared test, $P < 0.001$), and the ratios of the intermediate layers were significantly higher than those of the superficial layers (Chi-squared test, $P < 0.001$) in the left SC (A), right SC (B) and both sides of the SC (C). These results indicate that the disengagement-related neurons were located mainly in the deep layers. For the reward and attention shift-related neurons, the ratios of the deep layers as well as the intermediate layers were significantly higher than those of the superficial layers (Chi-squared test, $P < 0.001$) in the left SC (A), right SC (B) and both sides of the SC (C). There were no significant differences in the ratios between the deep and intermediate layers (Chi-squared test, $P > 0.05$). These results indicate that the reward and attention shift-related neurons were located mainly in both the deep and intermediate layers. For the visually-responsive neurons, the ratios of the superficial layers were significantly higher compared to the intermediate layers (Chi-squared test, $P < 0.001$), and the ratios of the intermediate layers were significantly higher compared to that of the deep layers (Chi-squared test, $P < 0.001$). Visually-responsive neurons were mainly located in the superficial layers.

Discussion

Visually-responsive SC neurons

The SC is a laminated structure, classically divided into superficial, intermediate, and deep layers. The superficial layers receive inputs directly from the retina and V1 (primary visual cortex), and project directly to the deeper layers of the SC and the pulvinar (May, 2006; Doubell et al., 2003; Hilbig et al., 2000). Consistent with the simple connections of the superficial layers, the visually-responsive neurons, which were located mainly in the superficial layers, responded to the contralateral light with shorter latencies (26.5 ms) than the other neuronal types. Previous studies reported that the mean response latency to visual stimuli was 21.4 ms in the superficial layers of the SC in Long Evans rats (Fortin et al., 1999), and 50 ms in albino rats (Thomas et al., 2005), which are comparable to our present findings. These neurons might transfer visual information to other types of SC neurons.

Reward and attention shift-related neurons

The reward and attention shift-related neurons, which responded to the contralateral CSs in the reward trials, were located mainly in both the intermediate and deep layers of the SC. The intermediate and deep layers of the SC have intimate connections with various cortical and subcortical structures and relatively few connections with the retina (May, 2006; Tardif and Clarke, 2002). One of the important functions of the SC is attention shifting (Goldberg and Wurtz, 1972; Gattass and Desimone, 1996). The “build-up” or “visuomotor” cells, which are recorded from the intermediate and deep layers of the monkey SC and involved in motor (saccade) preparation (Sparks and Hartwich-Young, 1989), are also involved in attention shifting (Kustov and Robinson, 1996; Ignashchenkova et al., 2004). The locations of the reward and attention shift-related neurons

in the present study correspond to the locations where electrical stimulation induces orienting behavior in rats (Sahibzada et al., 1986). The present results suggest that the reward and attention shift-related neurons in the rat SC might correspond to “build-up” or “visuomotor” cells in monkeys, and are involved in attention shifting.

Although the mean response latency of these neurons was longer than that of the visually-responsive neurons, some of these neurons had short latencies comparable to visually-responsive neurons, which are shorter than cortical neurons (Wang et al., 2014). These neurons might be involved in visual attention independent of the cortex. Consistently, SC inactivation induces behavioral impairments in a covert attentional task through mechanisms that are independent of the classic effects in the visual cortex (Zénon and Krauzlis, 2012). However, being part of the network of brain areas involved in spatial attention, the SC is also a node in descending pathways to guide behaviors to a target (Gandhi and Katnani, 2011; Borra et al., 2014). SC neurons with longer latencies might be controlled by these cortical outputs. These results are consistent with a previous study that found activity of monkey SC neurons in the intermediate and deep layers to be associated with both bottom-up and top-down shifts of attention (Bell and Munoz, 2008). Taken together, through the different (bottom-up and/or top-down) mechanisms, the rodent SC also guides behaviors to attended stimuli, and might output signals through its connections with the dopaminergic system (Redgrave and Gurney, 2006) and the predorsal bundle (Sahibzada et al., 1986).

Attention disengagement-related neurons

The attention disengagement-related neurons responded more strongly to the CSs requiring attention disengagement in sessions 3 and 4 than to other rewarding CSs in sessions 1 and 2, requiring no attention disengagement, although behavioral requirement was the same. The only

difference between the sessions 1 and 2 vs. sessions 3 and 4 was the lights; only one of the two lights was turned on in sessions 1 and 2 while two lights were simultaneously turned on in sessions 3 and 4. Therefore, differences in response magnitudes between sessions 1 and 2 vs. sessions 3 and 4 might be ascribed to differences in total luminance of the stimuli. However, this is unlikely since response magnitudes to the two lights in sessions 3 and 4 were significantly larger than response to the same stimuli in session 5, in which attentional disengagement was not required. Furthermore, we discovered that response latencies of attention disengagement-related neurons were faster and response magnitudes stronger specifically in the sessions 3 and 4 requiring attentional disengagement when compared to those in sessions 1, 2, and 5, in which lick latencies were faster than in sessions 3 and 4. These results strongly suggest that activity of attention disengagement-related neurons does not reflect simple motor-preparatory activity. In addition, activity of some attention disengagement-related neurons was negatively correlated with lick latencies in sessions requiring attention disengagement. Together, these results suggest that attention disengagement-related neurons specifically play a role in attentional disengagement processes to guide licking.

A previous human case study reported that a patient with lesions including the right SC showed deficits in saccades to the contralateral (left) target in an overlap condition requiring disengagement (Pierrot-Deseilligny et al., 1991). These human behavioral data are consistent with the present results in which activity of the attention desengagement-related neurons was associated with disengagement of attention from the ipsilateral light and attentional shift to the contralateral light. Although no previous neurophysiological studies have reported neurons associated with attentional disengagement, the frontal eye fields and parietal lobe, which are implicated in attentional disengagement by behavioral and EEG data in humans (Posner et al., 1984; Rivaud et al., 1994; Csibra et al., 1997), send projections to the deep layer of the SC (Sparks and

Hartwich-Young, 1989), the location where the attention disengagement-related neurons were found in the present study. Further studies are required to investigate whether activity of attention disengagement-related SC neurons reflects cortical activity.

Several studies suggest deficits in disengagement of visual attention as a unique feature of autism in young children (Rodier, 2000; Landry and Bryson, 2004; Elsabbagh et al., 2009, 2013). These studies investigated orienting reactions of young autistic and non-autistic children who looked at 3 computer monitors in front of them. Once attention was first engaged on a fixation stimulus in the central monitor, a second stimulus was presented on either side, either simultaneously (overlap condition) or successively (gap condition). Reaction time to the peripheral stimuli was longer in autistic children in the overlap condition compared with non-autistic children. This experimental situation is comparable to the present study, suggesting that the deep layers of SC might be involved in the pathology of autism. Furthermore, some pathological changes in the SC were observed in human autistic patients and animal models of autism (Dendrinos et al., 2011; Kleinhans et al., 2011; Zhao et al., 2013).

Conclusions

The present study demonstrated the existence of rodent SC neurons that are comparable to those in monkeys (visually-responsive neurons, and reward and attention shift-related neurons), as well as a new type of SC neuron that has not been reported in previous studies (attention disengagement-related neurons). The visual attention system can be remained either engaged or disengaged (Fischer and Breitmeyer, 1987) and based on our current findings, we suggest the following neural mechanisms of attentional engagement and disengagement. Reward and attention shift-related neurons might be involved in the engagement process, while attention disengagement-related neurons might be involved in the disengagement process. To attend a

contralateral target, attention to the ipsilateral target must be initially disengaged (see above). To disengage attention from the ipsilateral CSs, the attention disengagement-related neurons in the ipsilateral SC might inhibit reward and attention shift-related neurons in the contralateral SC, which are involved in engagement of attention to the ipsilateral target, through the inhibitory tecto-tectal pathway (Goodale, 1973; Munoz and Istvan, 1998). Interestingly, activity of the attention-disengagement neurons seemed to be inhibited in response to the ipsilateral CSs in sessions 1 and 2 requiring no disengagement; response latencies were slower and response durations were shorter in response to the ipsilateral CSs (Fig. 4). These results can be interpreted as follows. When an ipsilateral target without disengagement is presented, contralateral reward and attention shift-related neurons are activated to engage attention to the ipsilateral target, and at the same time activity of the attention-disengagement neurons in the ipsilateral SC is suppressed so that these attention disengagement-related neurons do not inhibit the reward and attention shift-related neurons in the contralateral SC. Further studies are required to prove or disprove this idea.

On the other hand, in the present study we required a licking response from the rats and not a saccade. Previous studies reported SC involvement not only in eye movements (saccades), but also in hand control (Borra et al., 2014) and locomotor decisions (Felsen and Mainen, 2008). The present study provides additional evidence with respect to a role of the SC in motor control; the rodent SC is involved in guiding lick behaviors, especially in an attentional disengagement condition.

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Figure legends

Fig. 1. Experimental setup (A) and trial (B) and session (C) types for an attention-shift task.

A: Experimental setup. Rats were prepared for chronic recording by forming receptacles of dental cement to accept artificial earbars. Electrodes were implanted in the lateral hypothalamic area for intracranial self-stimulation (ICSS) of the medial forebrain bundle. The rat was trained to lick when the spout was automatically placed close to its mouth. Auditory and visual conditioned stimuli (CSs) were presented by a speaker above its head and a light in front of each eye, respectively.

B: Trial types. In each trial, the CS appeared for 1 s, followed by spout protrusion close to the mouth for 2 s. In the reward (a) but not nonreward (b) trials, rats could obtain an ICSS reward if the rats licked the spout.

C: Session types. The task included 5 sessions. In session 1, right light (frequent stimulus) was sequentially presented, and when the left light, an infrequent stimulus, appeared, the rat could acquire reward if it licked the spout. In session 2, left light (frequent stimulus) was sequentially presented, and when the right light (infrequent stimulus) appeared, the rat could acquire reward if it licked the spout. In sessions 3 and 4, right and left lights (frequent stimuli) were similarly presented, respectively. However, the infrequent stimuli appeared with the frequent stimuli. Therefore, in sessions 3 and 4, the rat must disengage attention from the frequent stimulus. Arrows show attention disengagement and subsequent direction of attentional shift. In session 5, tone (frequent stimulus) was sequentially presented, and when both right and left lights (infrequent stimuli) were simultaneously presented, the rat could acquire reward if it licked the spout. Note that the infrequent stimuli in session 5 were the same as those in sessions 3 and 4, but attentional disengagement was not required in sessions 3 and 4. R1–R5, reward trials in sessions 1–5; N1–N5, nonreward trials in

sessions 1–5.

Fig. 2. Comparisons of lick latencies in the reward trials among the five sessions.

Lick latencies were significantly longer in sessions 3 and 4 than other sessions. ***, significant difference from sessions 1, 2, and 5 (Bonferroni test after one-way repeated measures ANOVA, $P < 0.001$).

Fig. 3. An example of an attention disengagement-related neuron recorded from the right SC.

A: Raster displays of neuronal activity and summed histograms in response to each stimulus. R1–R5 represent neuronal responses to the infrequent CSs associated with reward, and N1–N5 represent neuronal responses to the frequent CSs associated with nonreward. Horizontal bars above the raster displays indicate the stimulus presentation periods (1.0 s). Vertical dotted line in each of the raster displays and histograms indicates stimulus onset. Calibration at the right bottom of the figure indicates the number of spikes per trial in each bin. Bin width, 50 ms.

B: Comparison of response magnitudes of the neuron shown in A to the CSs. This neuron responded stronger to the CSs in session 3 (R3). *, significant difference from the CSs in R1, R2, R4, and R5 (Tukey test, $P < 0.05$); ***, significant difference from the CSs in N1 to N5 (Tukey test, $P < 0.001$).

Fig. 4. Mean response magnitudes (a), latencies (b), and durations (c) of the attention disengagement-related neurons.

A: Attention disengagement-related neurons recorded from the left SC. The mean response magnitude to the CSs was stronger (a), the mean response latency was shorter (b), and the mean response duration was longer (c) in session 4 than other sessions. **, *, significant differences from

the CSs in the reward trials of other sessions (Bonferroni test, $P < 0.01$, 0.05, respectively).

B: Attention disengagement-related neurons recorded from the right SC. The mean response magnitude to the CSs was stronger (a), the mean response latency was shorter (b), and the mean response duration was longer (c) in session 3 than other sessions. **, *, significant differences from the CSs in the reward trials of other sessions (Bonferroni test, $P < 0.01$, 0.05, respectively).

C: Attention disengagement-related neurons recorded from both sides of the SC. The mean response magnitude to the CSs was stronger (a), the mean response latency was shorter (b), and the mean response duration was longer (c) in sessions 3 and 4 than other sessions. ##, #, significant differences from the CSs in the reward trials of sessions 1, 2, and 5 (Bonferroni test, $P < 0.01$, 0.05, respectively).

Fig. 5. Relationships between response magnitudes to the CSs and lick latencies in session 3 in the disengagement-related neuron shown in Fig. 3.

There was a significant negative correlation between the response magnitudes and lick latencies ($P = 0.019$, simple linear regression).

Fig. 6. An example of a reward and attention shift-related neuron recorded from the right SC.

A: Raster displays of neuronal activity and summed histograms in response to each stimulus. The neuron responded to the infrequent CSs in the reward trials, but not to the frequent CSs in the nonreward trials.

B: Comparison of response magnitudes of the neuron shown in A to the CSs. ***, significantly different from the CSs in N1 to N5 (Tukey test, $P < 0.001$); #, significantly different from the CSs in R2 and R4 (Tukey test, $P < 0.05$). Other descriptions are the same as for Fig. 3.

Fig. 7. An example of a visually-responsive neuron recorded from the left SC.

A: Raster displays of neuronal activity and summed histograms in response to each stimulus. The neuron responded to the all CSs that included right light regardless of the session and reward association.

B: Comparison of response magnitudes of the neuron shown in A to the CSs. ***, significant difference from the CSs that did not include the right light (Tukey test, $P < 0.001$). Other descriptions are the same as for Fig. 3.

Fig. 8. An example of an inhibitory responsive neuron recorded from the right SC.

A: Raster displays of neuronal activity and summed histograms in response to each stimulus. The neuron showed inhibitory responses to the all CSs that included left light in the reward trials.

B: Comparison of response magnitudes of the neuron shown in A to the CSs. ***, significant difference compared to the CSs in the reward trials of session 2 and all the CSs in the nonreward trials (Tukey test, $P < 0.001$). Other descriptions are the same as for Fig. 3.

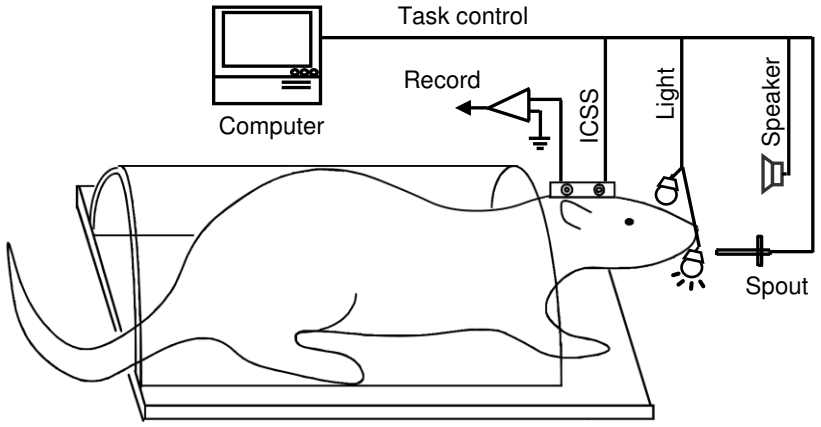
Fig. 9. Distributions of all neurons recorded from the SC.

A–C: Coronal sections, based on the atlas of Paxinos and Watson (2007). Values below each section indicate distance (mm) posterior from the bregma. DpG, deep gray layer; DpWh, deep white layer; InG, intermediate gray layer; InWh, intermediate white layer; Op, optic nerve layer; PAG, periaqueductal gray; SuG, superficial gray SC; Zo, zonal layer.

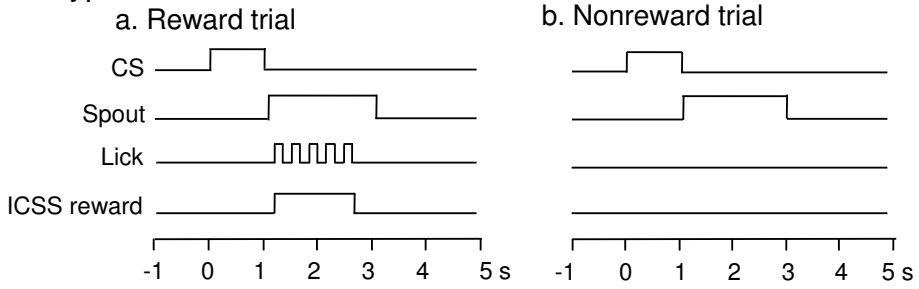
Fig. 10. Ratios of each neuronal type in the superficial, intermediate, and deep layers of the SC.

***, significant difference (Chi-squared test, $P < 0.001$)

A. Experimental setup



B. Trial types



C. Session types

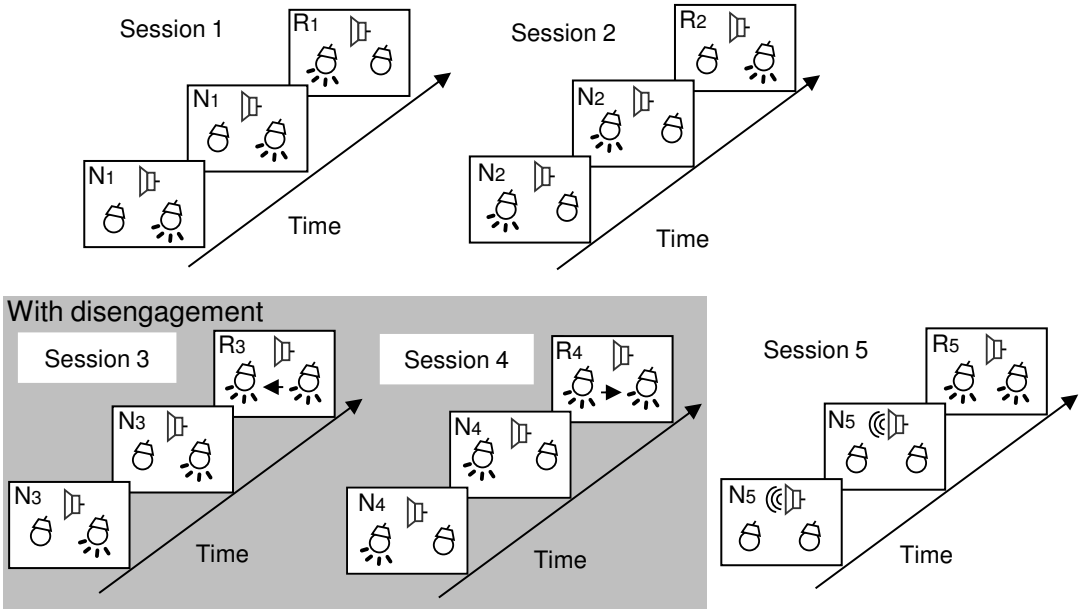


Fig. 1

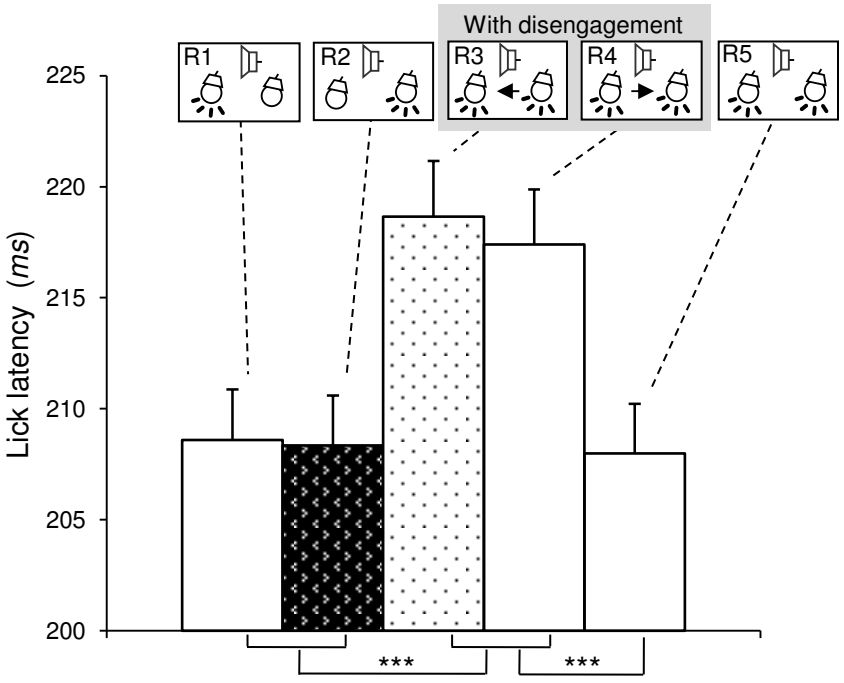

With disengagement
R1
R2
R3
R4
R5
225
220
215
210
205
200
Lick latency (ms)
***
***

Fig. 2

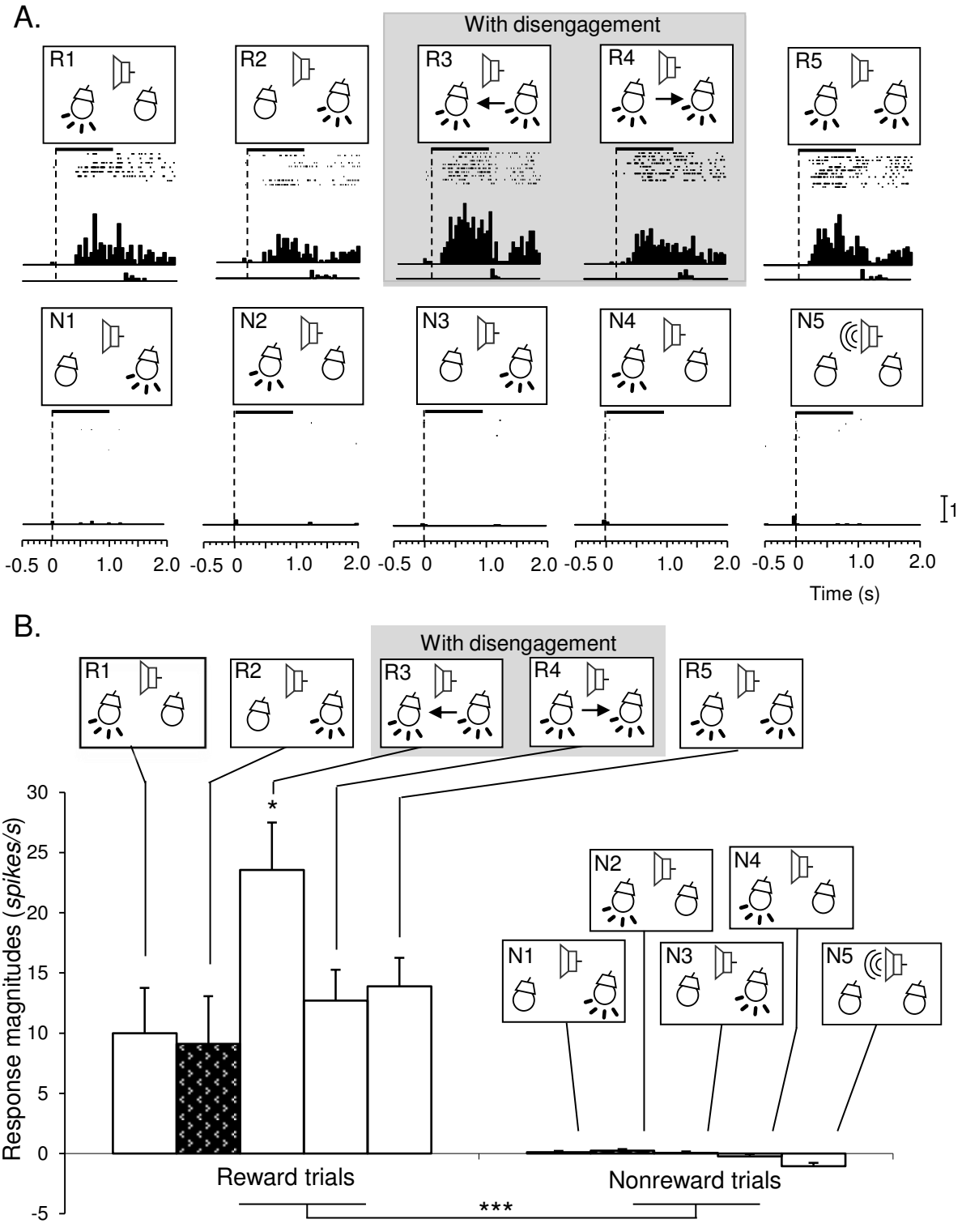

A.
With disengagement
R1
R2
R3
R4
R5
N1
N2
N3
N4
N5
-0.5 0
1.0
2.0
Time (s)
B.
With disengagement
Response magnitudes (spikes/s)
30
25
20
15
10
5
0
-5
*
Reward trials
Nonreward trials
***

Fig. 3

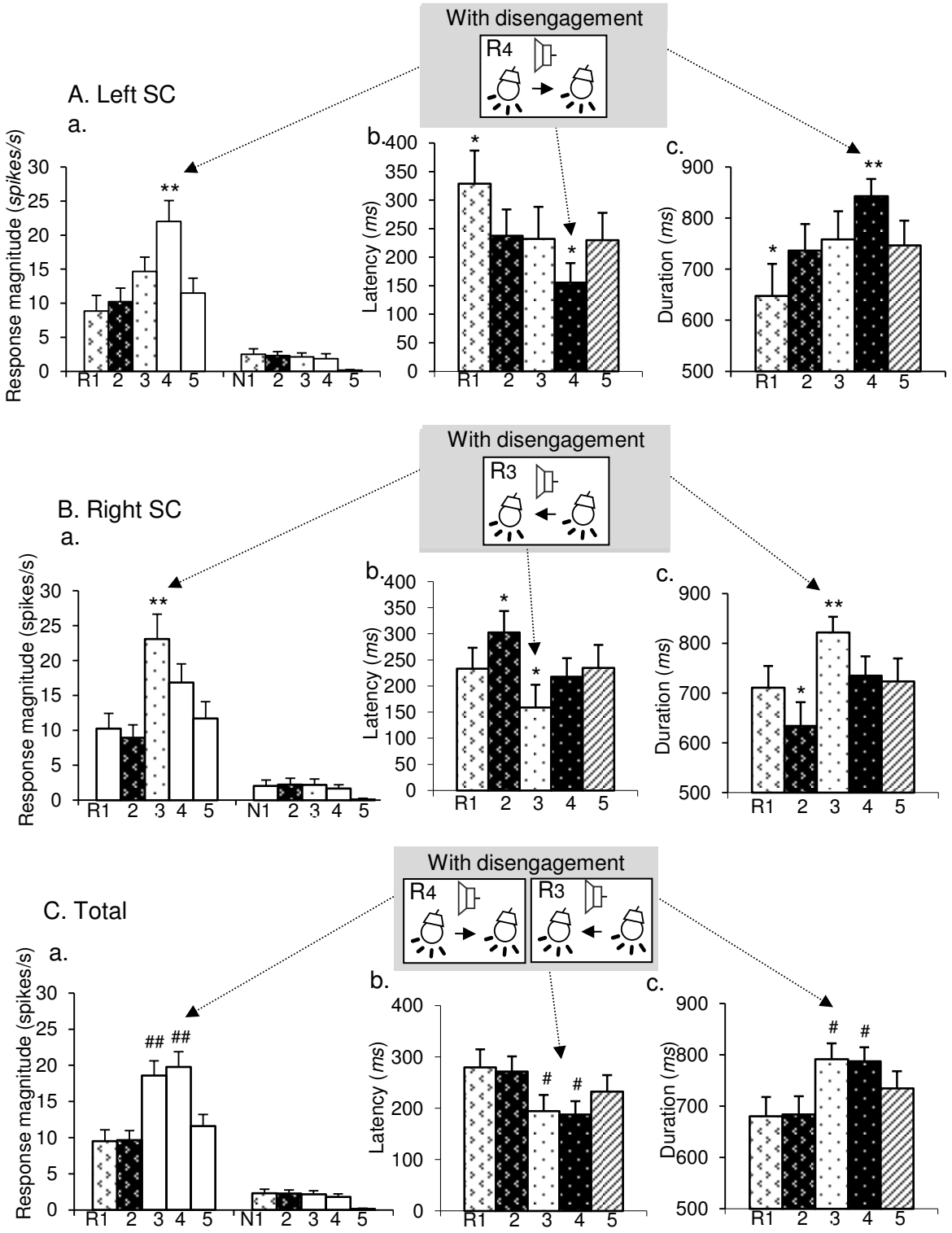

With disengagement
R4
A. Left SC
a.
Response magnitude (spikes/s)
b.
Latency (ms)
c.
Duration (ms)
R1 2 3 4 5 N1 2 3 4 5
With disengagement
R3
B. Right SC
a.
Response magnitude (spikes/s)
b.
Latency (ms)
c.
Duration (ms)
R1 2 3 4 5 N1 2 3 4 5
With disengagement
R4
R3
C. Total
a.
Response magnitude (spikes/s)
b.
Latency (ms)
c.
Duration (ms)
R1 2 3 4 5 N1 2 3 4 5

Fig. 4

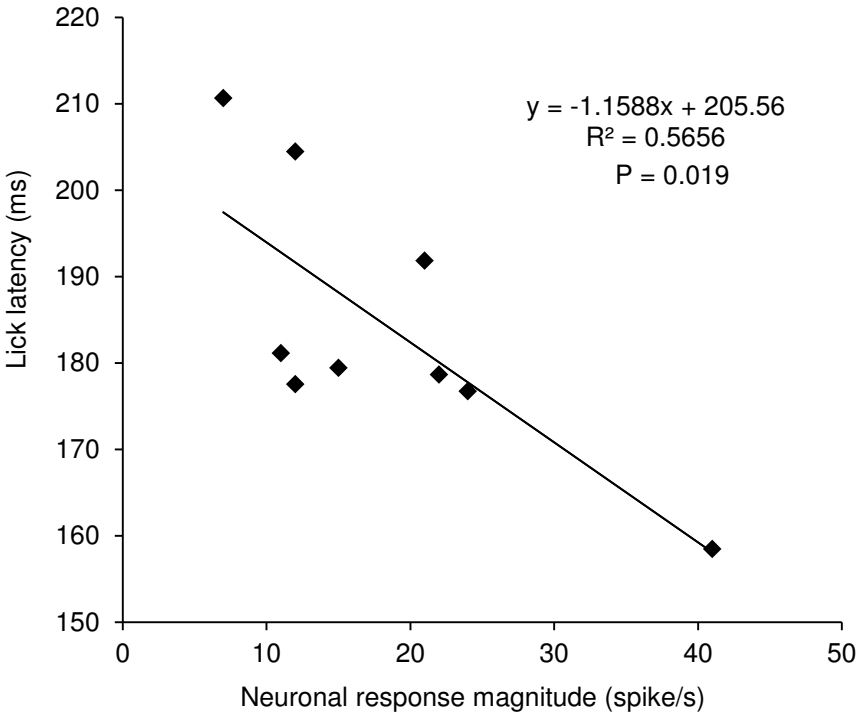

220
210
200
190
180
170
160
150
Lick latency (ms)
0
10
20
30
40
50
Neuronal response magnitude (spike/s)
y = -1.1588x + 205.56
R² = 0.5656
P = 0.019

Fig. 5

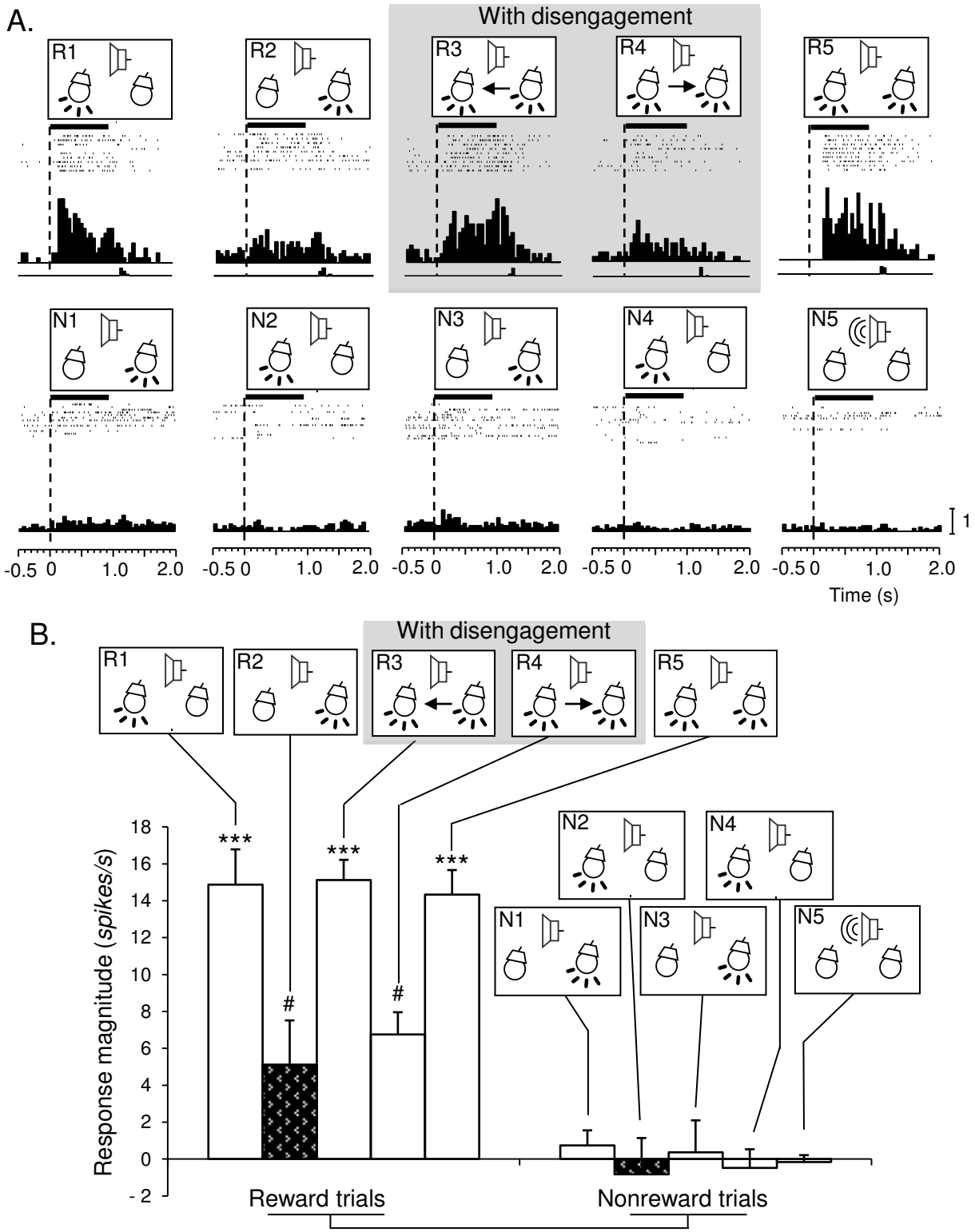
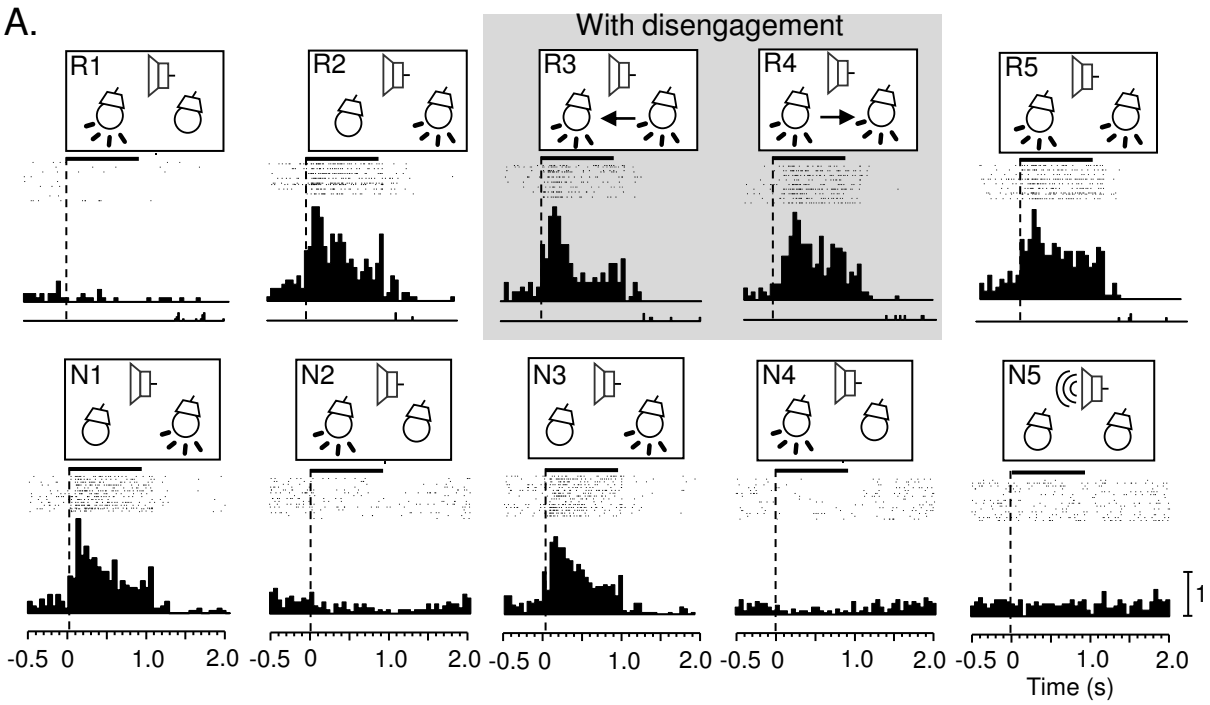

A.
With disengagement
R1
R2
R3
R4
R5
N1
N2
N3
N4
N5
-0.5 0
1.0
2.0
Time (s)
B.
With disengagement
Response magnitude (spikes/s)
18
16
14
12
10
8
6
4
2
0
- 2
***
#
Reward trials
Nonreward trials

Fig. 6


A.
With disengagement
R1
R2
R3
R4
R5
N1
N2
N3
N4
N5
-0.5 0
1.0
2.0
Time (s)

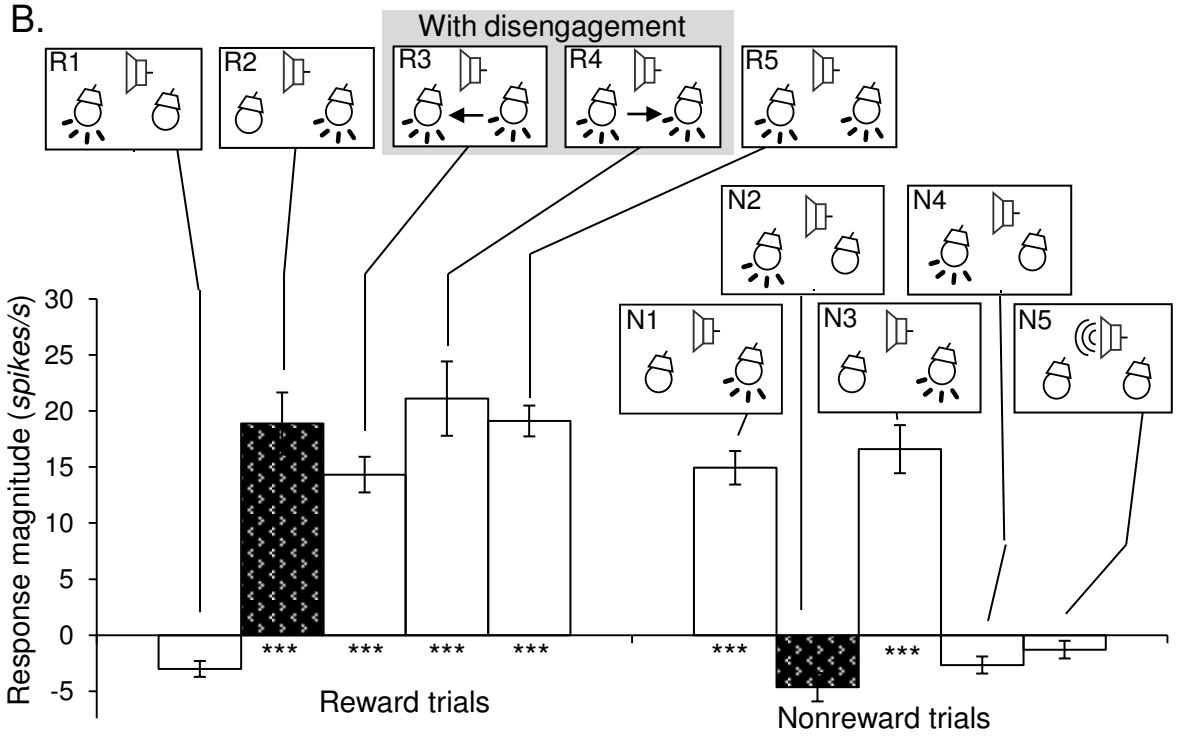

B.
With disengagement
R1
R2
R3
R4
R5
N1
N2
N3
N4
N5
Response magnitude (spikes/s)
30
25
20
15
10
5
0
-5
***
Reward trials
Nonreward trials

Fig. 7

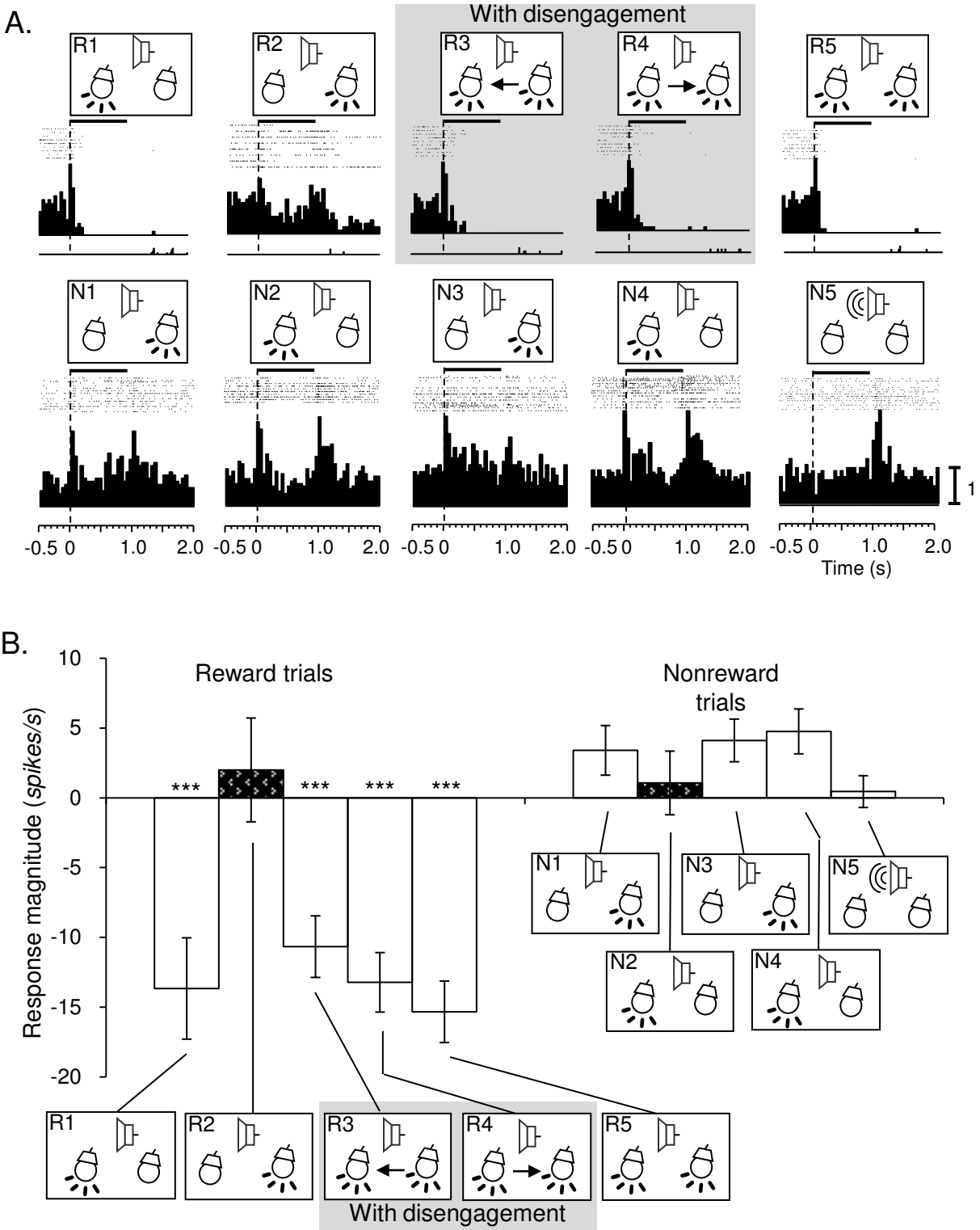

A.
With disengagement
R1
R2
R3
R4
R5
N1
N2
N3
N4
N5
-0.5 0
1.0
2.0
Time (s)
B.
Reward trials
Nonreward trials
Response magnitude (spikes/s)
10
5
0
-5
-10
-15
-20
***
With disengagement

Fig. 8

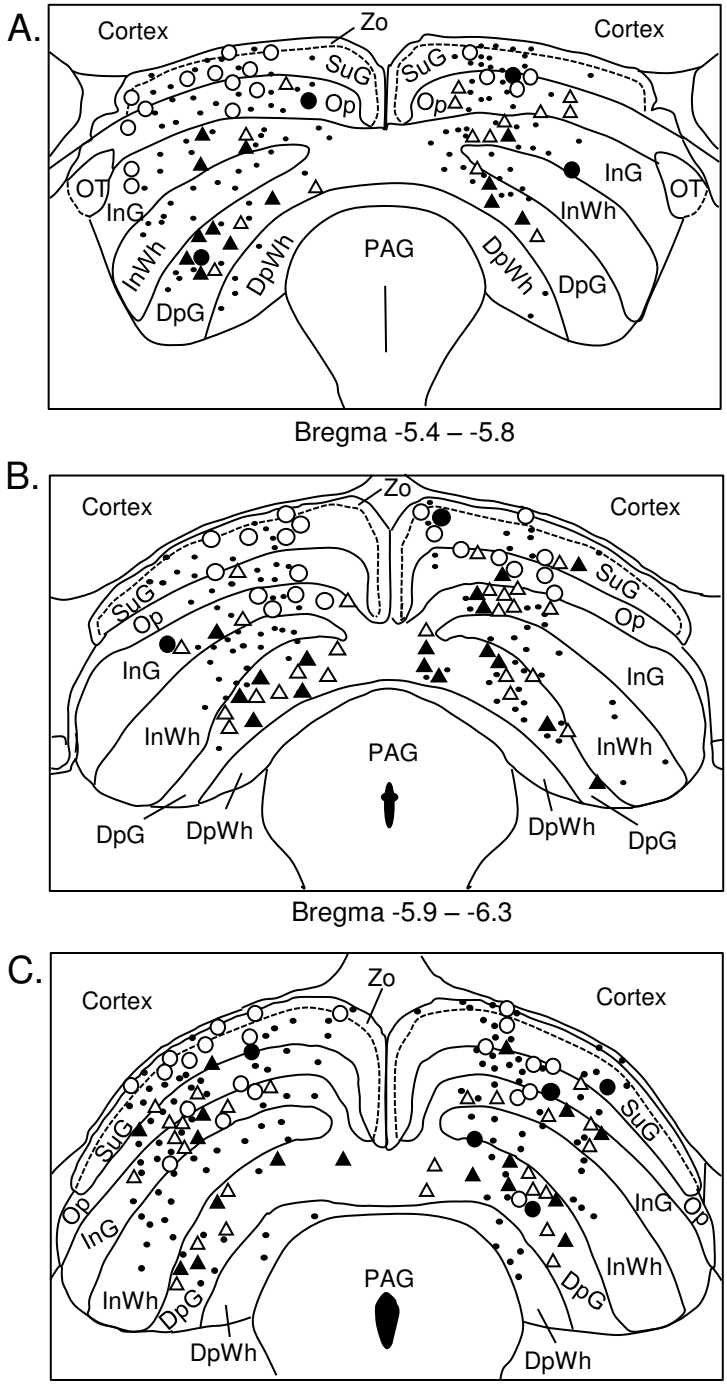

A.
Cortex
Zo
Cortex
SuG
SuG
Op
Op
InG
OT
OT
InG
InWh
InWh
PAG
DpWh
DpWh
DpG
DpG
Bregma -5.4 – -5.8
B.
Cortex
Zo
Cortex
SuG
SuG
Op
Op
InG
InG
InWh
InWh
PAG
DpG
DpWh
DpWh
DpG
Bregma -5.9 – -6.3
C.
Cortex
Zo
Cortex
SuG
SuG
Op
Op
InG
InG
InWh
InWh
PAG
DpG
DpG
DpWh
DpWh
Bregma -6.4 – -7.6
▲ : Attention disengagement-related
△ : Reward and attention shift-related
○ : Visually responsive
● : Inhibitory responsive
• : Non-responsive

Fig. 9

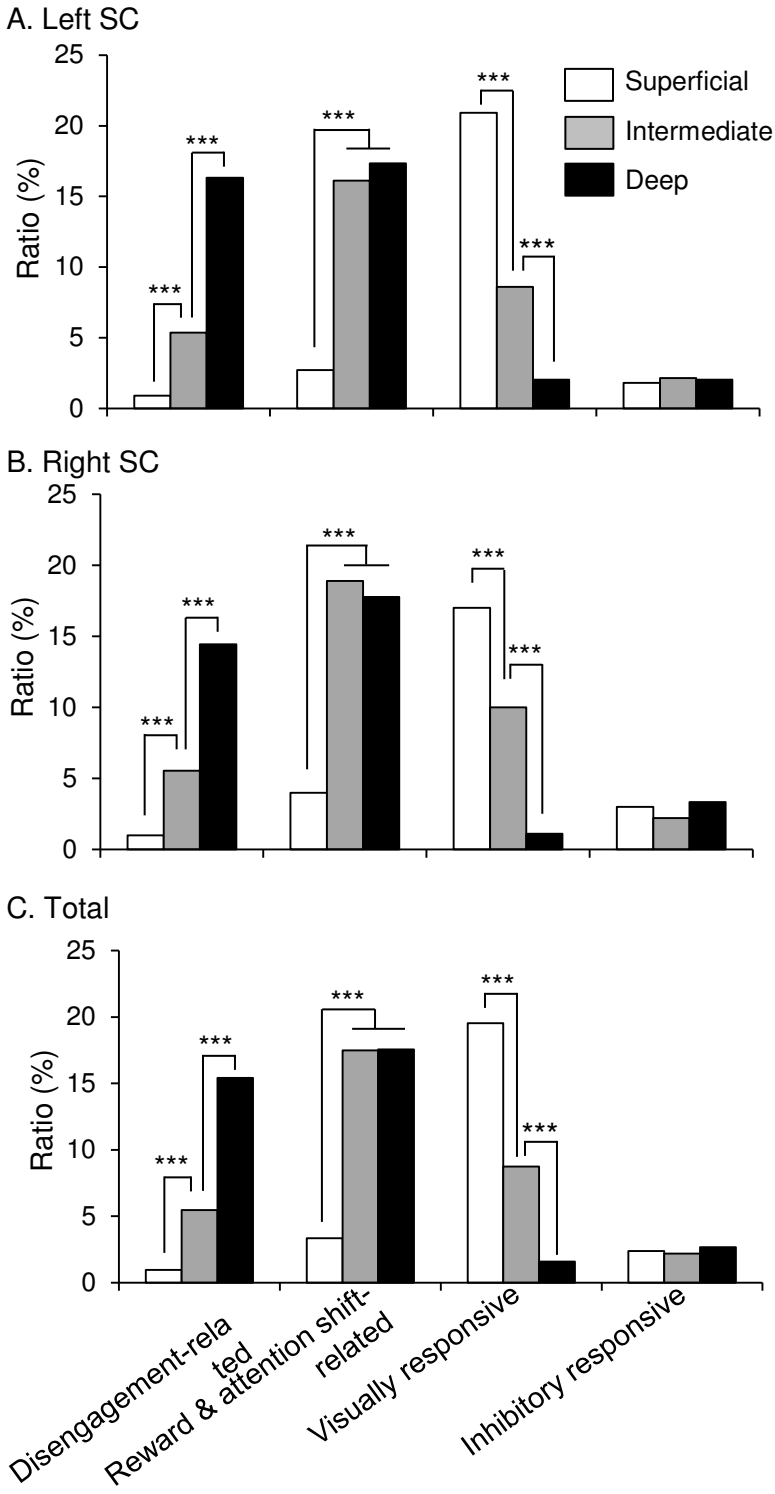

A. Left SC
B. Right SC
C. Total
Ratio (%)
25
20
15
10
5
0
***
Superficial
Intermediate
Deep
Disengagement-related
Reward & attention shift-related
Visually responsive
Inhibitory responsive

Fig. 10

Table 1. Classification and numbers of the SC neurons.

Classification	Number of neurons (R/L)			
	Superficial layers	Intermediate layers	Deep layers	Total
Disengagement-related	2 (1/1)	10 (5/5)	29 (13/16)	41 (19/22)
Reward and attention shift-related	7 (3/4)	29 (15/14)	34 (16/18)	70 (34/36)
Visually responsive	40 (18/22)	18 (9/9)	5 (3/2)	63 (30/33)
Inhibitory responsive	5 (3/2)	4 (2/2)	5 (2/1)	12 (7/5)
Total responses	54 (25/29)	61 (31/30)	71 (34/37)	186 (90/96)
No responses	158 (76/82)	120 (57/63)	119 (57/62)	397 (190/207)
Total	212 (101/111)	181 (88/93)	190 (91/99)	583 (280/303)

R, right superior colliculus; L, left superior colliculus. Numbers in parentheses indicate numbers of neurons in the right (R) and left (L) sides.