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Title	Competitor suppresses neuronal representation of food reward in the nucleus accumbens/medial striatum of domestic chicks
Author(s)	Amita, Hidetoshi; Matsushima, Toshiya
Citation	Behavioural Brain Research, 268, 139-149 https://doi.org/10.1016/j.bbr.2014.04.004
Issue Date	2014-07-15
Doc URL	http://hdl.handle.net/2115/60464
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Type	article (author version)
File Information	BBR_268_p139-.pdf

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Title: Competitor suppresses neuronal representation of food reward in the nucleus accumbens/medial striatum of domestic chicks.

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Acknowledgements: This study was supported by grant-in-aid for scientific research (Kakenhi) from the Japan Society for the Promotion of Science to TM (#22570070 and #25291071) and HA (#22/2260). We would also express our sincere gratitude to anonymous referees for their instructive and encouraging comments.

Abstract

To investigate the role of social contexts in controlling the neuronal representation of food reward, we recorded single neuron activity in the medial striatum/nucleus accumbens of domestic chicks and examined whether activities differed between two blocks with different contexts. Chicks were trained in an operant task to associate light-emitting diode color cues with three trial types that differed in the type of food reward: no reward (***S-***), a small reward/short-delay option (***SS***), and a large reward/long-delay alternative (***LL***). Amount and duration of reward were set such that both of ***SS*** and ***LL*** were chosen roughly equally. Neurons showing distinct cue-period activity in rewarding trials (***SS*** and ***LL***) were identified during an ***isolation*** block, and activity patterns were compared with those recorded from the same neuron during a subsequent ***pseudo-competition*** block in which another chick was allowed to forage in the same area, but was separated by a transparent window. In some neurons, cue-period activity was lower in the ***pseudo-competition*** block, and the difference was not ascribed to the number of repeated trials. Comparison at neuronal population level revealed statistically significant suppression in the ***pseudo-competition*** block in both ***SS*** and ***LL*** trials, suggesting that perceived competition generally suppressed the representation of cue-associated food reward. The delay- and reward-period activities, however, did not significantly different between blocks. These results demonstrate that visual perception of a competitive forager *per se* weakens the neuronal representation of predicted food reward. Possible functional links to impulse control are discussed.

Keywords: choice, impulsiveness, operant latency, pseudo-competition, social foraging (5 terms)

1. Introduction

Choice impulsiveness has been a topic of intensive experimental research in a wide range of behavioral sciences, including psychology [1, 2], psychopharmacology [3-6], behavioral ecology [7-10] and neuroscience [11-16]. To understand choice impulsiveness in a comprehensive manner, specifying the brain areas and networks that are specifically responsible for the choice of delayed reward is critical [17-19]. While the basal ganglia could play a pivotal role, their contribution to choice impulsiveness under natural conditions is not yet fully understood, primarily because an appropriate animal model has been lacking.

In a series of lesion experiments using domestic chicks, we have shown that localized lesions of the medial striatum (MSt) and nucleus accumbens (NAc) in the ventromedial basal ganglia enhance choice impulsiveness in 1-week-old domestic chicks [20] without affecting choices based solely on the amount of reward or the work-cost associated with the options [21]. Further, electrophysiological experiments in freely behaving chicks revealed that MSt/NAc neurons encoded the amount or proximity of reward during the initial cue period in which the color cue signaled the associated food reward [22]. MSt/NAc neurons with cue-period activity were also activated during delay and reward periods in which food reward was predicted and delivered [23]. However, a functional role for these multiple codes related to food reward remains elusive, except for an indirect suggestion that they might be involved in the computation of prediction error, as in cases of extinction [24]. Furthermore, a causal link between impaired coding of amount and proximity of reward in the MSt/NAc [22] and impulsive choice behavior in chicks remains unsubstantiated. Furthermore, neurons in isocortical areas (e.g., arcopallium in domestic chicks [25] and nidopallium

caudolaterale in pigeons [26,27]) also code aspects of anticipated food reward. Some of these isocortical neurons have been shown to represent integrated value in terms of temporally discounted food amount [26], thus could play an important role in the control of choice impulsiveness. To examine these issues, we turned to the ecological theory of kleptoparasitism [28] for natural and physiological conditions in which choice impulsiveness could be modulated using behaviorally tractable manipulations that were neither invasive nor pharmacological in nature.

Kleptoparasitism describes the stealing/exploitation of resources that have already been obtained by other conspecifics. Scramble kleptoparasitism is a special form of kleptoparasitism in which each food item is sharable among competitive foragers. Under these circumstances, animals differentiate their tactics into producers and scroungers according to the frequency dependence of fitness. For a producer, choosing a proximate food option is more profitable when being followed by a scrounging individual. This theoretical prediction has proven valid in domestic chicks. When compared with those trained in ***isolation***, chicks trained in competition gradually develop enhanced impulsiveness when forced to choose between a small amount of food following a short delay and a large amount of food following a long delay [29].

Although this finding initially seemed to match well with the collection-risk hypothesis [30-32], actual threat to food gain via competition proved unnecessary for development of choice impulsiveness. Even when chicks were physically separated from competitors and food scrounging did not occur, simply seeing a competitor that coincidently foraged nearby (presence of a potential competitor, or ***pseudo-competition***) caused an identical impulsive shift [29, 33]. It is to be stressed that subject chicks did not instantaneously change the choice impulsiveness in the context of

pseudo-competition, though operant peck latency was shortened as an acute effect [33]. Chicks had to be trained in the competitive social context consecutively for 1-3 days until they showed significantly higher impulsiveness in inter-temporal choice test performed in isolation. The social context thus does not directly modulate choices, but gradually contributes to the impulsiveness through processes and mechanisms that are not yet fully specified.

Here, as a step towards understanding the role of social context on the neuronal representations of food reward, we tested whether ***pseudo-competition*** could modulate cue-period activity in MSt/NAc neurons.

2. Materials and methods

2.1. Animals

Male chicks (*Gallus domesticus*, white leghorn) were used as recording animals (subjects) as well as companions. New hatchlings (post-hatch day 1) were purchased from a local supplier and housed in transparent cages (15 × 28 × 12 cm) in groups of two, and were placed in a thermo-controlled pen kept at ~26–30 °C under a 12-hour light:12-hour dark cycle starting at 08:00. On day 2, 3, and 4, chicks were fed with 1, 2, and 3 g of food per day (mixture of millet and mash food), respectively. Experiments began on day 5, and from then on chicks received 0.5–1 g of food during experiments and 4 g in the evening. Each day, chicks were moved to a training/experimental box (see 2.2, below), and returned to the home cage afterward. Water was freely available in the home cage. After completion of experiments, brains were dissected out after an overdose of anesthesia. In cases in which neuronal recordings were not made, chicks were euthanized by carbon dioxide. Experiments were conducted under the guidelines

and approval of the committee of animal experiments at Hokkaido University. These guidelines are based on the national regulation for animal welfare in Japan (law for the Humane Treatment and Management of Animals, after a partial amendment No.68, 2005).

2.2. Apparatus and training/test procedures

A thermo-controlled box (21 × 19 × 25 cm, maintained at 26–30 °C and illuminated by DC-powered light bulbs) was used for training and recording (see Fig. 1). The box was partitioned into two chambers (10 cm and 11 cm wide) by a Plexiglass wall and an electric liquid crystal shutter, so that subject chicks were physically separated from companions. In ***isolation*** (abbreviated as ***isol***), the shutter was turned on and the companion was not visible, while in ***pseudo-competition*** (abbreviated as ***comp***), the shutter was off allowing the subject and companion to see each other. Note that food was delivered separately, and as in our previous studies, food acquisition was not disturbed or altered in any way [26, 30]. On all trials in which the subject chick gained food, the companion simultaneously received 2 grains of millet.

The front panel of the subject's chamber was equipped with a pair of multi-color light-emitting diodes (LEDs) (3 cm apart and 5.5 cm above floor) and a corresponding pair of holes for response bars (1.5 cm below the LEDs). Pecks were recorded by microphones placed below the bars, and the sounds were stored together with neuronal signals. Three reward conditions were associated with three different LED color cues. No reward (***S-***) was indicated by red, small reward/small delay (***SS***, 1 grain, 0 s) by green, and large reward/large delay (***LL***, 6 grains, 1.5 s) by blue. A short tube at the center supplied millet food to the feeder. The companion's chamber was equipped with

a feeder, but not with LEDs or response bars.

Trials started with color-cue onset (time 0) followed by protrusion of the response bars after 0.5 s. After another 1 s, the cue was turned off and the response bars were withdrawn. Thus, the cue lasted 1.5 s, and the bars were protruded for 1 s. Food was supplied if the subject chick pecked at a response bar before it was retracted. The next trial began after a variable inter-trial interval ranging from 15–20 s that was not adjusted depending on the preceding trial type (see Fig. 1 and the time chart in Fig. 3A). Note that a brief mechanical lag (Δ = 0.2–0.3 s) inevitably occurred before the food was supplied. Based on our previous studies [29, 33], the delay for ***LL*** (or ***SS***) was fixed at 1.5 + Δ s (or Δ s) in all chicks studied, so that chicks could have chosen ***LL*** and ***SS*** options equally if tested in binary choices. It is to be noticed that we did not routinely examine chicks in binary choice tests and did not adjust the ***LL*** delay accordingly. Therefore, the subjective values of the ***SS*** and the ***LL*** options were not necessarily equal, rendering direct comparison of neuronal activities to be inappropriate between ***SS*** and ***LL***.

Training began on post-hatch day 5–7 after the subject had been habituated to the apparatus. Chicks were trained in two blocks (an ***isol*** block followed by a ***comp*** block) per day. Each block consisted of 80 pseudo-randomly arranged trials (20 ***LL***, 20 ***SS***, and 40 ***S-***). The sides of the LED and response-bar protrusion were counter-balanced. After consecutive training for 5 days (post-hatch day 11–14), most subject chicks were successfully trained to discriminate between rewarding (***SS*** and ***LL***) and non-rewarding (***S-***) options in binary choice tests. Chicks were accepted if they pecked in > 95% of the rewarding trials (both of ***SS*** and ***LL***) and in < 50% of the non-rewarding trials (***S-***) of the maintenance ***isol*** block. In binary choices between ***S-*** and one of the rewarding

options (***SS*** or ***LL***), these chicks chose the latter in > 95% of test trials. Binary choice tests between ***SS*** and ***LL*** were not accomplished except stated otherwise.

2.3. Chronically implanted electrodes, amplification of signal, and histological verification of the recording sites

After the task was learned, multi-wire electrodes were implanted under anesthesia using a ketamine/xylazine cocktail. A chick received an initial 0.4 ml i.m. injection of a 1:1 mixture of 10 mg/ml ketamine (Daiichi-Sankyo Co. Ltd., Tokyo, Japan) and 2 mg/ml xylazine (Sigma-Aldrich Japan Co., Tokyo, Japan). Supplementary doses of the same mixture (0.1 ml each) were injected if necessary. The chick was fixed to a rat stereotaxic apparatus modified to secure beaks. A bundle of four wires (25 μm thick, coated nichrome, AM System Co., USA) were vertically implanted into the ventromedial part of the telencephalon. The wires were inserted through a stainless-steel guide tube (o.d. 0.35 mm, i.d. 0.25 mm, 16 mm long; Small Parts Inc., USA) and fixed by superglue such that the bare tips of the wires protruded ~500 μm from the end of the tube. The tube was connected to a microdrive that was firmly fixed to the skull using dental resin. Coordinates of guide-tube insertion were 1.5–2.0 mm rostral to the fronto-parietal suture, 1.25–1.5 mm lateral from midline, and 5.0–7.0 mm below the brain surface. The implantation was not vertical, but was tilted 25° in the rostroventral direction. Before surgery, electrode tips were plated in 0.9% NaCl solution to reduce resistance to 100–500 kΩ.

The four wires were connected to a four-channel head stage amplifier, and two of these four signals were selected for extracellular recording through an AC-coupled differential amplifier (cut-off frequency at 0.3 kHz, amplification of × 2,000) with a

band-pass filter (0.5–1.5 kHz, 18 dB per octave). The signal was A/D converted at a sampling rate of 20 kHz through a Power1401 interface, and sorted and stored using Spike2 software (CED Co., UK) on a Windows PC. The sorted neurons were further verified using Offline Sorter (Plexon Co., USA) and those with poor separations and low signal-to-noise ratios were discarded. Unitary data accepted as single neuron activity were processed for generating rastergrams and peri-event histograms using MATLAB (version R2010b, MathWorks, USA). Bin width was set at 100 msec in histograms. Further statistical analyses were made using R (version 2.6.0, Windows version), a PC language developed for statistic computation.

Chicks quickly recovered from anesthesia and started to actively eat food on the day of surgical operation. Recording sessions usually started from the next day. Every day, chicks received at least two blocks for maintenance training, namely an ***isol*** block followed by a ***comp*** block, even when no units were encountered. Neuronal activities were recorded after the pre-operative performance (see above in 2.2.) was confirmed in the ***isol*** block. In search of neurons with sufficiently large spike amplitude (with signal-to-noise ratio > 2), electrode was advanced once or twice a day by around *ca.* 75-300 µm each.

Recording sessions continued until the electrode was advanced by 2-3 mm in total, or for 1-3 weeks after the surgery. In some cases, however, we stopped recording earlier when neurons were no more available. After recording sessions were completed, chicks were given a trans-cardiac perfusion of a fixative (4% paraformaldehyde in 0.1 M PB) under deep anesthesia induced by 0.8 ml of ketamine/xylazine mixture. The skull was post-fixed for 3–4 days in the same fixative at 4 °C, and then the brain was dissected out, embedded in yolk, and cut into 50-µm-thick sections using a microslicer. Slices were

mounted on Amino-Siliane coated slide glasses, and dried and stained in cresyl violet. Recording sites were reconstructed based on the stereotaxic atlas [34] and the reformed nomenclature for the avian brain [35].

2.4. Recording and analysis of neuronal activity

When an isolated neuron was encountered, we started to record its activity in single-option trials in which ***SS***, ***LL,*** or ***S-*** cues were presented singly. The liquid crystal shutter was initially turned on, and the activity was recorded in an ***isol*** block that consisted of 80 pseudo-randomly arranged trials (20 ***LL***, 20 ***SS***, and 40 ***S-***). The sides of the LED and response bar protrusion were counter-balanced. In some neurons, we also recorded the activity in binary-choice trials (10 ***LL***/***SS*** trials) randomly mixed in the ***isol*** block. In these probe trials, ***SS*** and ***LL*** were simultaneously presented and chicks freely chose one of the two options. We stopped recording if the spike shape changed considerably or if the cell was lost. If recording was successfully maintained until the end of the ***isol*** block, we stopped the task, turned off the liquid crystal shutter and restarted recording neuronal activity in a ***comp*** block. In some neurons, we successfully recorded a second ***isol*** block if spike shape was stable.

2.4.1. Statistical characterization of recorded neurons

After a recording session was over, generalized linear model (GLM) analysis was conducted for each neuron to examine whether the recorded activity was cue-related. The firing rate (spikes/s) recorded in rewarded trials of the ***isol*** block was fitted by four categorical variables representing periods of interest within the trial: inter-trial, cue, delay, and reward (Fig. 3A, horizontal bars). These periods were defined as follows relative to cue onset (time 0): inter-trial, -5.0 to 0 s (duration, 5 s); cue: 0 to 0.2 s (0.2 s);

delay: 1.5 to 1.7 s (***SS***) or 2.9 to 3.1 s (***LL***) (0.2 s); reward: 1.9 to 2.9 s (***SS***) or 3.4 to 4.4 s (***LL***) (1 s). Spikes occurring between 1.7 and 1.9 s (***SS***) or between 3.2 and 3.4 s (***LL***) were excluded. Data from trials in which the chick failed to peck the bar were excluded.

Because firing rate is thought to follow a Poisson distribution, a logarithm was adopted as the link function to convert the spike rate to the linear predictor ***X***, which was estimated by a linear function of ***period*** (cue, delay, and reward, as reference to the inter-trial interval):

$$\boldsymbol{X} = \beta_0 + \beta_1 \times \boldsymbol{period.}$$

The neuron was assumed to have significant cue-period related activity if the estimated co-efficient (β_1) had a significant non-zero value for ***period*** = cue, and if the probability that the β_1 value included 0 was low enough ($P < 0.01$). Further statistical analyses were conducted on the data from neurons that showed significantly high cue-period activity in the ***isol*** block.

2.4.2. Statistical analyses of the effects of pseudo-competition on normalized neuronal activity

2.4.2.1. Normalization of cue-, delay- and reward-period activity by inter-trial (baseline) firing

Baseline firing rates were measured during the last 5 s of the inter-trial intervals for all trial types (***S-***, ***SS,*** and ***LL***) and averaged for each neuron. The population baseline firing-rate was compared between ***isol*** and ***comp*** blocks using paired *t*-test at $P < 0.05$. For cue, delay, and reward activity, the firing rate was first normalized by dividing it by the averaged baseline firing-rate of each neuron, and converted by a common logarithm such that 0.0 represented baseline activity and a value of 1.0 would indicate a firing rate 10 times that of baseline.

2.4.2.2. Statistical comparison between blocks in individual neurons

Those neurons that showed a significant difference between blocks in the cue-period were searched for on the basis of the normalized neuronal activity. We applied Mann-Whitney's U-test between ***isol*** block (20 trials) and ***comp*** block (20 trials) for each of ***LL*** and ***SS***. Significance level was set at $P < 0.05$.

2.4.2.3. Statistical examination of the trial order effect using GLM

When a neuron was encountered, we initially characterized its activity pattern in ***isol*** block, and thereafter examined if the firing could change in ***comp*** block. The order of blocks was fixed, and the difference between the blocks could be ascribed to the order of trials, or to the social context that differed between the blocks. To dissociate these possibilities, we applied a *post-hoc* statistical analysis using generalized linear model (GLM) in each neuron. Any gradual changes due to repeated trials could be detected as trial effect, whereas effect of different social contexts will be detected as block effect.

In reference to the data recorded in the 20 trials of ***isol*** block, the inter-trial (baseline) firing rate and the normalized cue-period activity was converted to Z-score, such as:

Z-score = {(firing rate in each trial) – (mean of the firing rate in 20 trials)}
/ {estimated standard deviation of the firing rate in 20 trials}

Thereafter, assuming that the Z-score follows a Gaussian distribution, we estimated the Z-score to represent predictor ***X*** by a linear function of ***trial*** (1 to 40) or that of ***block*** (***isol*** and ***comp***) in the following 3 formulae:

Model (null): $\boldsymbol{X} = \gamma_0$

Model (trial): $\boldsymbol{X} = \gamma_0 + \gamma_1 \times \boldsymbol{trial}$

Model (block): $\boldsymbol{X} = \gamma_0 + \gamma_2 \times \boldsymbol{block}$

A full model including both ***trial*** and ***block*** was not considered, because these two

parameters are mutually dependent. For each of these models, AICs (Akaike Information Criteria) were calculated and significance of the estimated coefficient (γ_1 and γ_2, $P < 0.05$) was examined. We concluded the effect of social context rather than the trial order effect, when the Model (block) had smaller AICs than the other two models.

2.4.2.4. Statistical comparisons between blocks at the level of neuronal population

Statistical comparisons were also conducted between blocks at the population level. The normalized neuronal activity was compared using a two-way, repeated-measures ANOVA with factors of block (***isol*** or ***comp***) and trial type (***S-***, ***SS***, and ***LL*** for cue activity, ***SS*** and ***LL*** for delay and reward activity). Significance level was set at $P < 0.05$.

3. Results

3.1. Behavioral effects of pseudo-competition

To confirm the acute behavioral effect of pseudo-competition reported previously [33], latencies of the first operant peck at the response bar were compared in 19 chicks between ***isol*** and ***comp*** blocks in ***SS*** and ***LL*** trials (Fig. 2). Onset of the first peck was measured from the time at which the response bar was protruded. A repeated-measures ANOVA revealed a significant main effect of block (***isol*** vs. ***comp***; $F_{1,18} = 31.70$, $P < 0.001$), but not of trial type (***SS*** vs. ***LL***; $F_{1,18} = 3.23$, *NS*) or the interaction ($F_{1,18} = 0.00$, *NS*). We therefore conclude that the chicks pecked more quickly in the ***comp*** block than in the ***isol*** block in both ***SS*** and ***LL*** trials. Although the latencies did not significantly differ between blocks in some individual chicks, we included neurophysiological data

obtained from these chicks in the analyses.

(Figure 2 around here)

3.2. Neuronal activity in cue, delay, and reward periods

3.2.1. Categorization of neurons

Unitary spike activity was recorded from 130 neurons in 37 chicks. Of these, 45 neurons were successfully tested in both ***isol*** and ***comp*** blocks, with 37 of them having significant cue-period excitation in the ***isol*** block as determined by the off-line GLM analysis. Intervening pause period between the blocks was 6.0 ± 0.5 min (mean ± sem, n=37), whereas duration of the recording blocks was 25.3 ± 0.7 min (***isol*** block) and 26.5 ± 0.6 min (***comp*** block), respectively. As shown in Fig. 3E, recording sites were localized in bilateral MSt/NAc. The following analyses are based on these 37 neurons.

Neurons were further categorized based on whether they had distinct delay- and/or reward-period activities (Fig. 3B). Typical examples are shown in Fig. 3C and D. In some neurons, initial phasic excitation in the cue period (arrows 1) was followed by inhibition (Fig. 3C) or excitation (3D) during the operant period. Notice that the cue-period activity was higher in rewarding trials (***SS*** and ***LL***) than in non-rewarding trials (***S-***) in these examples (also see Fig.7B for population data).

3.2.2. Activities in inter-temporal choices

In some neurons, second characteristic waves of activity arose in the delay period just before food delivery (arrows 2, Fig. 3C) or during the reward period (arrows 3, Fig. 3D). Neuronal activity was compared between single-option trials and binary-choice trials in arbitrarily selected 11 neurons, and significant delay-period activity was found in 6 of these 11 neurons. Fig. 4 shows an example recoded from the same neuron as in

Fig. 3C. Among 10 inter-temporal choice trials (bottom), the chick chose ***SS*** rewards three times and ***LL*** rewards seven times. Early delay-period activity (indicated by green arrows) arose when an ***SS*** reward was chosen, whereas late activity (blue arrows) arose when ***LL*** rewards were chosen. Delay-period activity was thus dependent on the chosen option. On the other hand, the cue-period activities did not clearly differ between these two sets of trials.

In these 11 neurons tested in the inter-temporal choices (namely in 11 ***isol*** blocks of recording), average number of ***LL*** choices among 10 test trials was 5.4 ± 0.7; binomial test revealed $P = 0.3438$ for the null hypothesis that assumed equal choices between ***SS*** and ***LL***. The difference of delay between ***LL*** and ***SS*** (1.5 sec) is thus supposed to have balanced two options. In the subset of 6 neurons with significant delay-period activity, firing rate was measured during the delay-period of ***SS*** trials and during the comparable period of ***LL*** trials (a gray square in Fig. 3A). Wilcoxon signed-rank test (two-tailed) applied to these 6 pairs of activities revealed $P = 0.03125$, indicating a significantly higher firing rate in ***SS*** trials than in ***LL*** trials. It is suggested at the population level that the MSt/NAc neurons coded the temporal proximity of the chosen reward.

(Figures 3 and 4 around here)

3.3. Changes of cue-period activity in pseudo-competition

3.3.1. Normalized cue-period activity in individual neurons

The cue-period firing rate was normalized by dividing by the inter-trial (baseline) firing rate of each block. For each of ***SS*** and ***LL*** trial types, the normalized activity was compared between the blocks by using Mann-Whitney's U-test in individual neurons. Significant difference was found in 8 out of the 37 neurons (Fig.5D), and two examples

with distinct changes are selected and shown in Fig. 5A and B. Of these 8 neurons, 7 neurons showed suppression in either or both of ***SS*** and ***LL*** trials (with 2 overlapping neurons), whereas enhancement in ***LL*** trials occurred in one neuron. Significant effects were not found in the remaining 29 neurons.

In Fig. 5C, the normalized firing rate and inter-trial (baseline) firing are shown as respective Z-score plotted against the trial number. In both neurons, the block model gave rise to smaller AICs than the trial model. In the neuron #31 (Fig. 5A), for example, AICs were 164.4 (null model), 154.9 (trial model), 142.8 (block model) for ***LL*** trials, and 177.1 (null model), 173.3 (trial model), 166.6 (block model) for ***SS*** trials. It is thus concluded that the cue-period activities showed an abrupt suppression in the ***comp*** block, and the significant difference found between the blocks was not due to gradual changes after repeated trials. It is also to be noticed that the baseline firing underwent a significant step-like decrease in ***comp*** block. Similar effect was found also in neuron #22 (Fig. 5B), in which the baseline firing underwent a step-like increase instead.

Of the 8 neurons with significant inter-block differences, GLM model comparisons revealed smaller AICs in the trial model in 7 neurons. Of these 7 neurons, inter-trial (baseline) firing rate was compared between blocks using Mann-Whitney's U-test. The baseline rate was significantly lower in ***comp*** block in 1 neuron and higher in 5 neurons, whereas it did not show significantly differ in 1 neuron. On the other hand, the trial model showed a smaller AICs value in the remaining 1 neuron. In this exceptional case (neuron #6, categorized as one of the 4 neurons with significant changes only in ***LL*** trials), AICs were 149.4 (null model), 143.4 (trial model), 146.5 (block model) for ***LL*** trials, indicating that firing rate underwent a gradual change, which may not be an effect of different social contexts.

Out of the 37 neurons analyzed, 6 neurons thus showed a stepwise suppression (and 1 neuron enhancement) as an effect of ***pseudo-competition***. In these 6 neurons, 5 showed suppression in ***LL*** trials, and 3 neurons in ***SS*** trials with 2 overlapping neurons (Fig. 5D). No clear relationship was found between the changes in the ***comp*** block and their delay/reward-period activities (Fig. 3B).

3.3.2. Population-level analyses on cue-, delay-, and reward-period activity

We also examined whether significant changes occurred at the level of neuronal population. The normalized firing rate was averaged over the 37 neurons, and compared across contexts as superimposed traces (Fig. 6). Although significant block effect was found in relatively small number of neurons as described above, the averaged traces showed considerable differences.

We applied repeated-measures ANOVA for individual neurons to the normalized firing rate in the cue-period. First, we compared the baseline firing-rate (Fig. 7A), and found no significant difference between ***isol*** and ***comp*** blocks (n = 37 neurons; t_{cal} = -0.77, *d.o.f.* = 36; NS, paired t-test). Cue-, delay-, and reward-period activity was then normalized by dividing each by the respective baseline firing-rate (Fig. 7B–D).

(Figures 6 and 7 around here)

Analysis of cue-period activity (Fig. 7B) revealed significant main effects for both block (***isol*** or ***comp***) and trial (***S-***, ***SS***, or ***LL***) types (block type: $F_{1,36} = 29.70$, $P < 0.001$; trial type: $F_{2,72} = 36.30$, $P < 0.001$), but no significant interaction among them ($F_{2,72} = 0.67$, *NS*). In contrast, analysis of delay- and reward-period activity (7C and 7D) revealed a significant main effect of trial (***SS*** or ***LL***), but not block type, and no interactions (delay-period: block type, $F_{1,36} = 3.72$, *NS*; trial type, $F_{1,36} = 19.70$, $P < 0.001$; interaction $F_{1,36} = 0.41$, *NS*; reward period: block type, $F_{1,36} = 0.008$, *NS*, trial

type, $F_{1,36} = 15.5$, $P < 0.001$; interaction, $F_{1,36} = 0.88$, *NS*).

In some cases, a second ***isol*** block was performed after the ***comp*** block. Among the 37 neurons, 11 neurons were recorded under these conditions, and they showed a partial recovery during the cue period. However, comparison of population cue-period activity by ANOVA revealed that this recovery was suggestive but not significant, whereas the effect of trial type remained significant, as did the lack of interaction (block type: $F_{1,10} = 4.10$, *NS with* $P = 0.07$; trial type: $F_{2,20} = 6.42$, $P = 0.007$; interaction: $F_{2,20} = 1.63$, *NS*).

4. Discussion

4.1. Sequential representation of different aspects of food reward

MSt/NAc neurons characteristically represent different aspects of food reward in a sequential manner. Understanding how these neurons represent foraging decisions step-by-step can provide insight into whether social context modulates neuronal representation of food.

4.1.1. Cue-period activity codes value of predicted food based on memorized associations

Within a fraction of a second after the LED was lit, MSt/NAc neurons responded to the cue color with phasic excitatory activity (Figs. 3 and 6). The activity significantly differed depending on trial type (Fig. 7B), suggesting that it encoded factors of the food reward associated with each of the color cues. In a previous study [22], we included a fourth option (a color cue associated with a large reward/short delay, or ***LS***) and found that some MSt/NAc neurons had higher cue-period activity in both ***LS*** and ***SS*** trials than they did in ***LL*** trials (thus encoding the short delay), while others had higher activity in both ***LS*** and ***LL*** trials than they did in ***SS*** trials (thus encoding the large

reward). This suggested that cue-period activity represents factors such as the temporal proximity and amount of the associated food reward. Indeed, similar representations of proximity and amount have been reported in the ventral striatum of rhesus monkeys [36] and the NAc of rats [37]. Further, recent research has revealed a code for delay-discounted value of food rewards in primate NAc neurons [38], suggesting that these factors are integrated into subjective value at the single neuron level as has been reported in nidopallium caudolaterale of pigeons [26] (Fig. 8).

In apparent contrast, and consistent with our previous reports [20,21], MSt/NAc neurons also fired during the cue-period of ***S-*** trials in which reward was not expected, albeit at lower rates than in rewarding trials (Fig. 3C,D and 6). Upon LED onset, irrespective of whether the color was rewarding or not, subject chicks generally oriented to the apparatus (particularly to the feeder), even after they had been trained for several weeks. Because most chicks did not peck the bar in trials in which a pair of non-rewarding options (two red LEDs) were presented, assuming that chicks falsely predicted food in ***S-***trials is not reasonable. Another weak possibility is that cue-period activity directly contributes to the sensorimotor control of orienting behavior and execution of operant pecks, as has been suggested in neurons of the arcopallium [25] that project to the MSt/NAc [39]. Under this theory, the shortened operant peck latencies in the ***pseudo-competition*** block (Fig. 2) would be a direct consequence of suppressed cue-period activity. However, this idea is not plausible given that our previous lesion experiments failed to cause any detectable changes in the latencies or performance of pecks at the response bar or food [21,24]. Furthermore, we have not found a correlation between activity of MSt/NAc neurons and pecking actions [22,23]. The suppressed cue-period activity is therefore not likely to be causally linked to the

observed shortening in peck latency.

4.1.2. Delay-period activity codes proximity of predicted food

In many MSt/NAc neurons, a characteristic excitation appeared in both types of rewarding trials immediately after the cue LED was turned off and the response bar was withdrawn (Fig. 3-6). As the delay-period activity rose according to the subject's own choice (Fig. 4), we may argue that the activity is downstream to the decision mechanism that remains unspecified. Furthermore, the interval-timing mechanism postulated in the pallio-striatal neural network [27,40] may intervene between the decision and delay-period activity (Fig. 8). Similar gradual increases in firing rate (or climbing ramp activity) have also been reported in monkeys [41,42], rats [43] and pigeons [27] preceding food delivery. While the delay-period activity may be involved in behavioral self-control by allowing the subject to wait patiently for future food, this theory is not compatible with the finding that MSt/NAc lesions made chicks stay even longer at the empty feeder in extinction blocks [24]. An alternative possibility is that the delay-period activity contributes to the timing of an internal clock, and its impairment slowed down the subjective passage of time.

Another explanation is that some delay-period activity was a non-specific response to the LED offset and/or bar withdrawal. For example, the subtle sound of the feeding instrument could have cued these transient activities. However, these possibilities also seem unlikely given that the slowly developing ramp activities cannot be ascribed to external stimuli (see Fig. 3C, 6, particularly in ***LL*** trials).

4.1.3. Reward-period activity codes actual food

Representation of gained reward has been repeatedly documented in chicks [23] and rats [44-46] in terms of changes in activity (either excitatory or inhibitory) that

appear when reward is delivered. In probe extinction trials, for most neurons, these activity changes immediately vanished [45], supporting the idea that the reward-period activity represents actual reward. Currently, neither the specific aspects of the reward that could be responsible for the activity (visual perception, food intake action, gustatory signals, or any combination of these), nor the functional role of these changes in controlling foraging behavior are known. However, a plausible explanation is that representations of actual food rewards in this area are used as prediction-error signals that are then represented in the midbrain dopaminergic neurons [47], a supposition supported by a lesion study that resulted in impaired extinction of operant pecks in chicks [24].

4.2. Functional roles for the social modulation of MSt/NAc neurons

In this study, we found that cue-period activity of some MSt/NAc neurons was lower in the block of ***pseudo-competition*** than in the preceding ***isolation*** block. The suppression in the cue period could be due to different social context, or caused by repeated trials. Results of our GLM analysis were supportive for the former in many of the neurons with significant difference, however effect of trial repetition predominated in one neuron. Some neurons were tested in a second ***isol*** block, but recovery remained suggestive. In future studies, recordings must be accomplished with a reversed block order, such that the neuronal activities are tested first in the ***pseudo-completion***.

It is also to be noticed that the inter-trial (baseline) firing rate underwent a considerable difference between blocks, and the suppressed cue-period activity was often accompanied by an increased baseline firing. We assume that phasic change relative to baseline firing plays a role as signal on representation of food, however, it is

an open question as to (1) how the baseline firing is controlled, and (2) how the baseline firing contributes to the representation of food reward in MSt/NAc.

The following two possibilities should be considered as explanations for the suppressed cue-period activity observed in this study.

4.2.1. Suppressed critic signal leads to impulsiveness

Value representations in the mammalian ventral striatum have been proposed to function as a learning critic, with the output signal from the ventral striatum being used to update the policy adopted by a separate actor [43,48-51]. Acute suppression of the cue-period activity found in this study may represent a suppressed critic signal, which gradually causes an impulsive bias in the actor signal. The dorsal striatum has been proposed to convey actor signals in mammals [49,50,52], which is homologous to the lateral striatum in birds. Neural activity in the lateral striatum of chicks should be examined for lasting effects resulting from selective suppression of MSt/NAc neurons.

4.2.2. Suppression due to distracted vigilance

Another scenario is warranted because the one mentioned above does not account for all the observations. First, it does not explain the suppressed cue-period activity that was observed in the ***S-*** trials of the ***pseudo-competition*** block. Second, occurrence of suppression in both ***SS*** and ***LL*** trials (Fig. 5D, 6 and 7) is also counter-intuitive as an account for the development of impulsive choices biased for the ***SS*** option [29,33]. Here we theorize that a competitively foraging chick can distract the subject from attending to the LED cues, and that the cue-period activity represents attention to cues. To test this hypothesis, future studies should include a condition in which the subject's vigilance is distracted by a non-social stimulus such as a predator or novel objects. We should also examine whether such non-social distractions also lead chicks to commit more choices

that are impulsive.

4.3. Neural connections possibly responsible for the representations of food

Considerable neuroanatomical studies have so far been accumulated on the afferent and efferent connections of the avian MSt/NAc. Here, we discuss neural substrates possibly involved in the assumed mechanism and function of social modulation.

4.3.1. Afferent connection to MSt/NAc

The sequential representations of different aspects of food reward described above could be due to convergent inputs to the MSt/NAc [39,53]. Tract tracing studies have so far revealed inputs from a wide range of pallial structures (*i.e.*, avian homologues of isocortex such as the arcopallium and nidopallium) [54], the subpallial thalamus [55], and neuromodulatory nuclei including the ventral tegmental area (VTA) and substantia nigra (SN) for dopaminergic afferents [56-59], and the raphe nuclei in the pons for serotonergic afferents [60].

To date, functional significance has been specified in some of these areas including the arcopallium, nidopallium, VTA, and raphe nuclei. Single unit recording of the arcopallium [25] revealed neuronal representations of predicted reward in cue and delay periods of an operant task as well as in response to unexpected food reward. We may therefore assume that the arcopallium is a source that signals actual reward and color-reward associations directly to the MSt/NAc. Whether neuronal activity in the arcopallium can be modulated by pseudo-competition or other non-social distractors is another question that can be asked, However it is unlikely given that bilateral lesions of the arcopallium failed to cause choice impulsiveness, but rather made chicks work-cost averse and enhanced the preference to easy food [21]. On the other hand, neuronal

coding of delay-discounted value of delayed food has also been documented in the nidopallium caudolaterale (Ncl) [26]. In this context, results of recent pharmacological studies using dopamine D1 receptor antagonist in pigeons [61,62] are highly suggestive, as they clearly dissociated the functional roles of dopaminergic actions between Ncl and striatum. As to the dopaminergic afferents from VTA and SN, however, their functional role in impulse control remains unclear, except for an indirect suggestion that dopamine could play a permissive role in synaptic potentiation in the MSt/NAc of chicks [63]. Afferents from the raphe nuclei should also be considered, because a systemic application of fluvoxamine (selective serotonin reuptake inhibitor) made chicks commit less impulsive choices [6].

4.3.2. Efferent connections from MSt/NAc

The brain areas to which these MSt/NAc signals are sent and processed for controlling choices also remain unknown. Recently, projection patterns in bird MSt/NAc subdivisions have been documented in detail [64], revealing divergent projections to ventral pallidum, lateral hypothalamus, the preoptic area in the diencephalon, and reciprocal connections to VTA, substantia nigra pars compacta (SNc), raphe nuclei, and surrounding regions such as the midbrain reticular formation.

The functional significance of these efferent pathways is only partially understood. The MSt/NAc efferents to VTA and SNc may convey a reward signal that is used to compute prediction error signals as discussed above. The prediction error signals in VTA and/or SNc could be socially modulated by the presence of a potential competitor, leading to a gradual change in the actor system assumed in the lateral striatum. Possible involvement of dopaminergic neurons in the developmental control of impulsiveness should be examined in the future.

5. Conclusions

Cue-period activity in the MSt/NAc was suppressed in the ***pseudo-competition*** block, suggesting that the neuronal representation of predicted food reward is modulated during social foraging. Because the cue-period activity differed among trial types (***S-***, ***SS*** and ***LL***), we conclude that neuronal representation of the food value is suppressed. We theorize that lowered attention to the reward cues could underlie the observed social suppression. While the observed suppression during rewarding trials was consistent with changes in behavior (shortening of peck latencies), causal links between them remain unproven. Delay- and reward-period activity were not modulated at the neuronal population level, suggesting that different aspects of foraging behavior (self-control, interval timing, and actual food gain) are represented in a sequential manner. A tentative scenario is proposed in which suppression of food value leads to gradual development of choice impulsiveness through a cumulative experience of competition. Impaired vigilance attention may be responsible for the suppressed cue-period representation of food value.

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Figure legends

Fig. 1 Schematic diagram illustrating the task. A multi-color LED cue (blue, red, or green) located on the right or left side of the front panel was turned on, and response bars were subsequently presented below the LED 0.5–1.5 s after cue onset. Upon successful pecking at the bar, millet food was delivered after a short delay through the feeding tube at the center. When tested in isolation, the liquid crystal shutter was turned on, so that the subject chick (yellow) did not see the companion chick (gray) next door. When tested in pseudo-competition, the shutter was turned off, and the companion chick was visually exposed. Notice that the companion was given two grains of millet in every trial in which the subject gained food. The inlet table indicates the association between the LED color and the reward type. A brief and variable mechanical lag (Δ = 0.2–0.3 s) was inevitable. ***S-*** indicates color cue signaling no reward, ***SS*** and ***LL*** indicate small reward/short-delay and large reward/long-delay options, respectively.

Fig. 2 Behavioral effect of pseudo-competition. In the pseudo-competition block (***comp***), operant peck latency was significantly shorter than during the isolation block (***isol***) in both ***SS*** and ***LL*** trials, but no significant difference was found between ***SS*** and ***LL*** trial types. Horizontal bars represent mean latencies for each chick (n = 19). See text for statistics.

Fig. 3 Cue-, delay-, and reward-period activity in MSt/NAc neurons.

A: Procedure of *SS* and *LL* trials; vertical dashed lines indicate cue onset (0 s), delivery of *SS* food (1.7 s) and *LL* food (3.2 s), respectively. Inlet horizontal bars indicate the

cue-, delay- and reward-periods in which action potentials were analyzed (see Fig.7). In some neurons, firing rate was measured also during the period just after the response bar was withdrawn (gray square) in LL trials. B: The 37 units with significant cue-period excitation were categorized according to the activities in delay and/or reward period. C and D: Typical examples of single-unit activities shown in rasters (above) and averaged histogram (below), with superimposed traces of action potentials (inlet). Bin width was set at 100 ms in this and following figures. Recordings are aligned at the cue onset (0 s). In both cases, the cells transiently fired at the cue onset (arrows 1), and subsequently fired prior to food delivery (neuron C; arrows 2) or after food delivery (neuron D; arrows 3). Activities during the operant period were either inhibitory (C) or excitatory (D), and not correlated with each peck. E: Histologically reconstructed sites of the neurons were plotted on chick brain atlas; anterior-posterior levels (A9.0 to A10.2) follow the atlas by Kuenzel and Masson (1988). Circles denote neurons with significant cue-period excitation (n=37). H: hyperpallium, M: mesopalium, N: nidopallium, E: entopallium, lSt: lateral striatum, mSt/NAc: medial striatum and nucleus accumbens, GP: globus pallidus, tsm: tractus septo-mesencephalicus.

Fig. 4 Delay period activity represented chosen option. Activities in single option trials (top), binary choices between rewarding (***SS*** or ***LL***) and non-rewarding (***S-***) options (middle), and inter-temporal choices between ***SS*** and ***LL*** (bottom) were compared. The cell depicted here is the same cell as shown in Fig. 3A. Underlines indicate the chosen option. In the inter-temporal choice trials, the delay-period activity (green arrows) occurred only when ***SS*** was chosen.

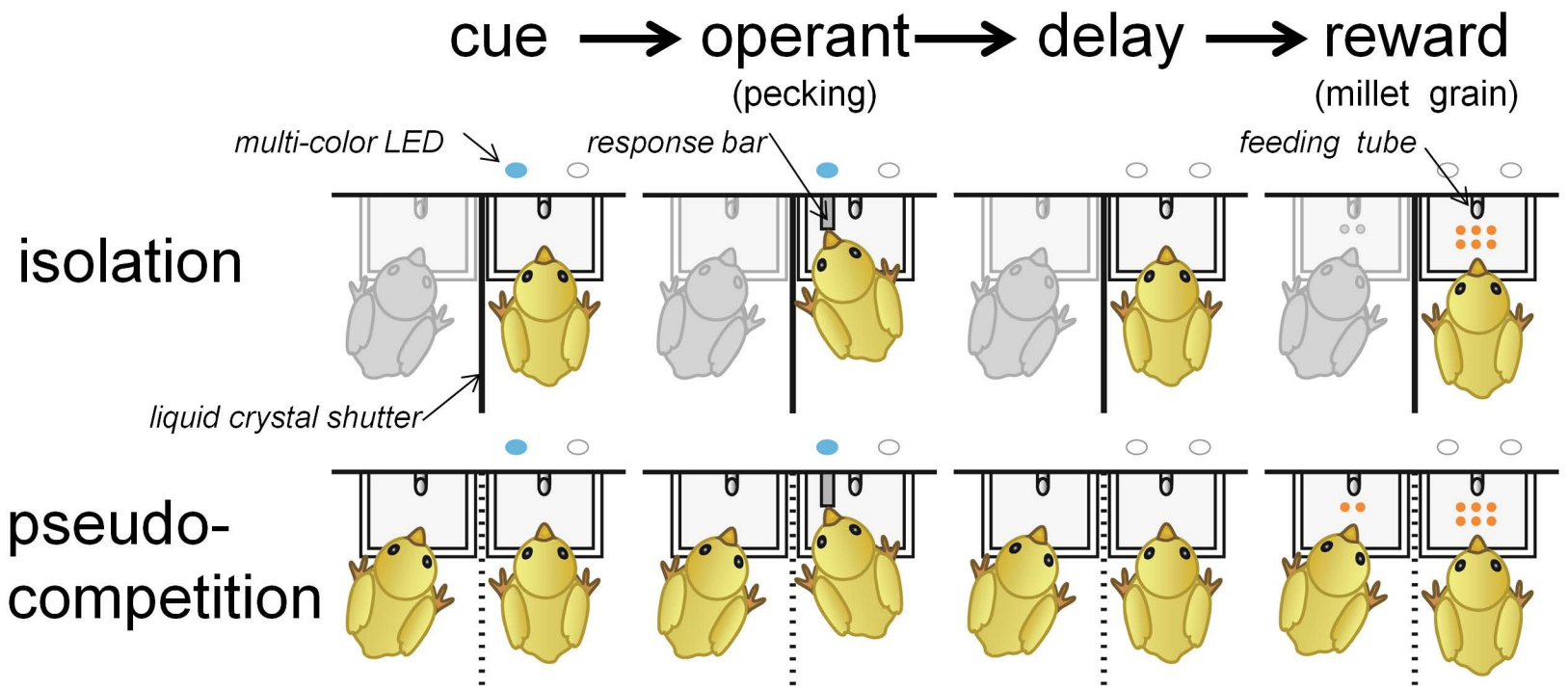
Fig. 5 Competition suppressed cue period activity in the population of MSt/NAc neurons; analyses of individual neurons. A and B: Neurons #31 (A) and #22 (B) represented cases with the most distinct suppression of normalized cue-period activities in *SS* and *LL* trials. The firing rate was normalized by dividing by the inter-trial (baseline) rate. Note suppression and enhancement in the inter-trial (baseline) firing rate in the neuron #31 (A) and #22 (B), respectively. C: The normalized cue-period activities were further converted to Z-score in reference to the 20 trials in isolation block (Y-axis), and plotted against the number of trials (X-axis). Trials 1-20 were recorded in the isolation block, and those 21-40 in the subsequent pseudo-competition block. Note that the changes occurred immediately from the first trial of the pseudo-competition block. D: Normalized firing rate showed significant difference between the blocks in 8 neurons; 7 showed suppression and 1 enhancement. The examples #31 and #22 represent the cases selected from those with significant suppression in both *LL* and *SS* trials (n = 2*). On the other hand, one of the 4 neurons (n = 4) with suppression in LL trials (neuron #6) represent trial order effect. See text for further statistical analyses.

Fig. 6 Competition suppressed cue period activity in the population of MSt/NAc neurons; superimposed averaged traces. Normalized firing rates were averaged over 37 neurons and are superimposed for ***isol*** and ***comp*** for each trial type (***S-***, ***SS,*** and ***LL***).

Fig. 7 Competition suppressed cue period activities in the population of mSt/NAc neurons; quantitative analyses. A: Baseline firing rate in inter-trial intervals (5-s period immediately preceding cue onset) did not significantly differ between isolation (***isol***) and pseudo-competition (***comp***) blocks. Horizontal bars indicate mean firing rates

of the recorded cells with excitatory cue-period activity (n = 37). **B** (cue-period): Significant differences were found among trial types (***S-***, ***SS,*** and ***LL***), and between blocks (***isol*** and ***comp***). No significant interactions were found. **C** (delay-period) and **D** (reward-period): Significant differences were found in firing rates between ***SS*** and ***LL***, but not between ***isol*** and ***comp***. No significant interaction was found.

Fig. 8 MSt/NAc neurons as a platform for sequential representation of different aspects of food rewards. See text for explanations. Distinct neuronal activities arise sequentially in M/NAc neurons after external events (such as color cues and food delivery) and internal events (recall, value, decision, interval-timing, and reward perception). Pseudo-competition selectively suppresses the cue-period activity. Behavioral consequences of the suppression remain to be studied.



	LED color	amount (grains)	delay (sec)
S-	red	0	-
SS	green	1	0+Δ
LL	blue	6	1.5+Δ

Figure 1

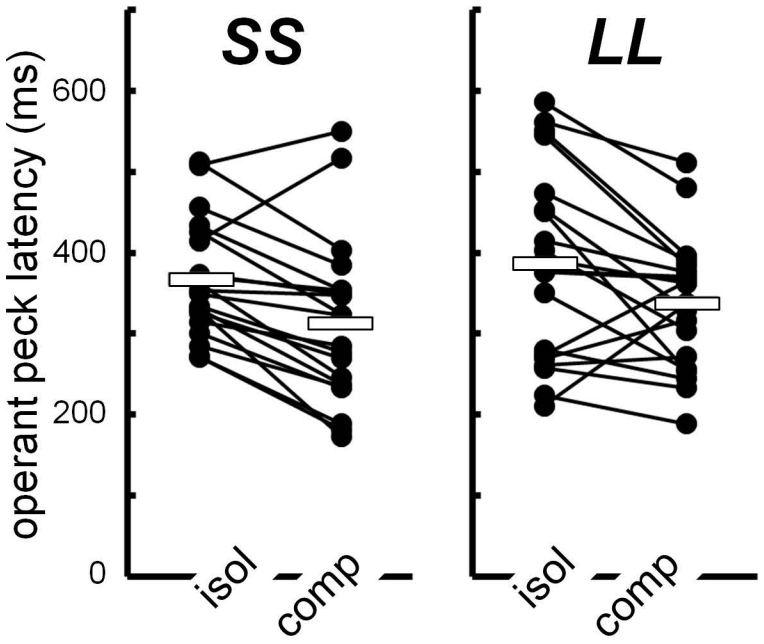

SS
LL
operant peck latency (ms)
600
400
200
0
isol
comp
isol
comp

Figure 2

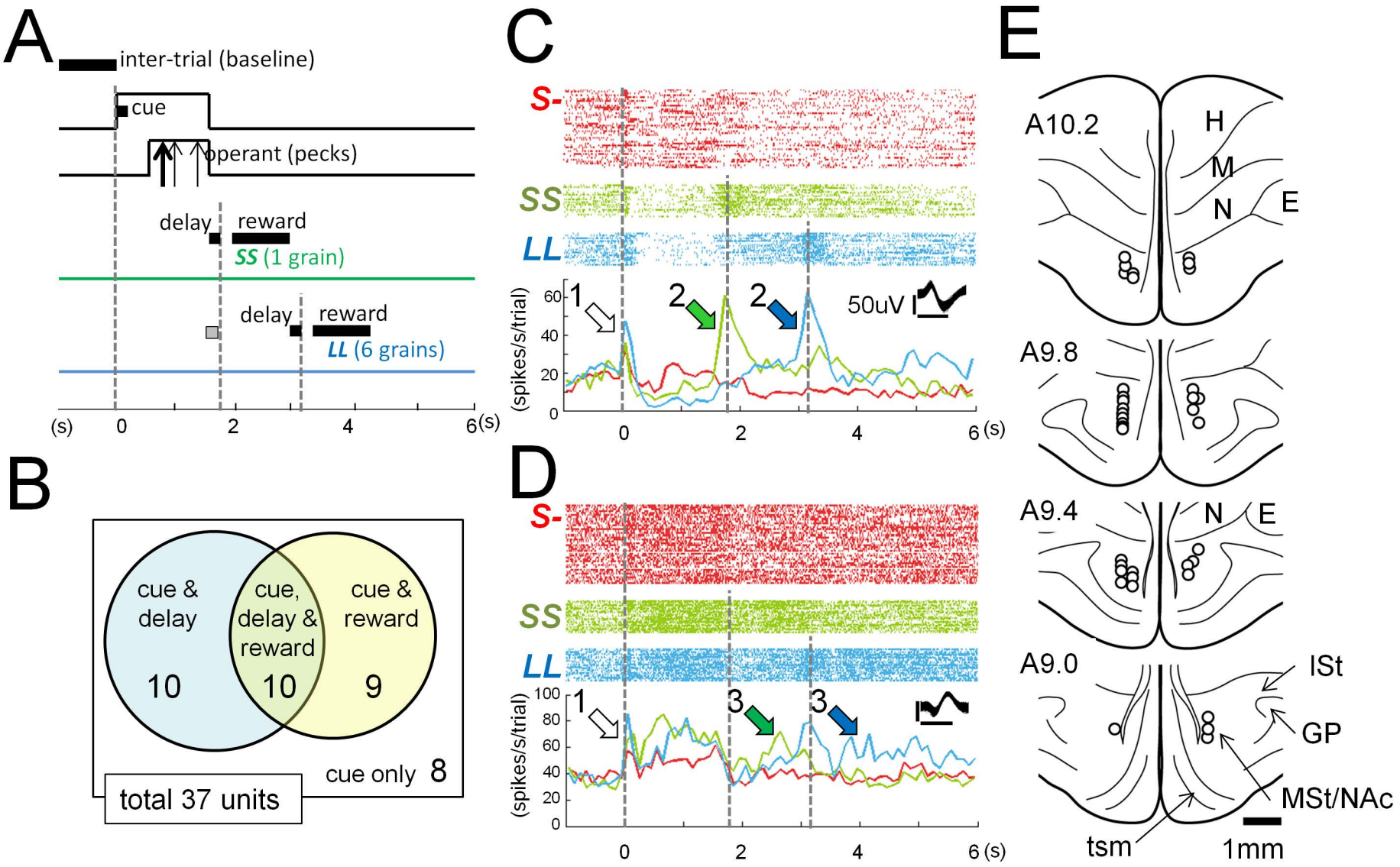

A
inter-trial (baseline)
cue
operant (pecks)
delay
reward
SS (1 grain)
delay
reward
LL (6 grains)
(s)
0
2
4
6(s)
B
cue & delay
10
cue, delay & reward
10
cue & reward
9
cue only 8
total 37 units
C
S-
SS
LL
(spikes/s/trial)
60
40
20
0
1
2
2
50uV
0
2
4
6 (s)
D
S-
SS
LL
(spikes/s/trial)
100
80
60
40
20
0
1
3
3
0
2
4
6 (s)
E
A10.2
H
M
N
E
A9.8
A9.4
N
E
A9.0
lSt
GP
MSt/NAc
tsm
1mm

Figure 3

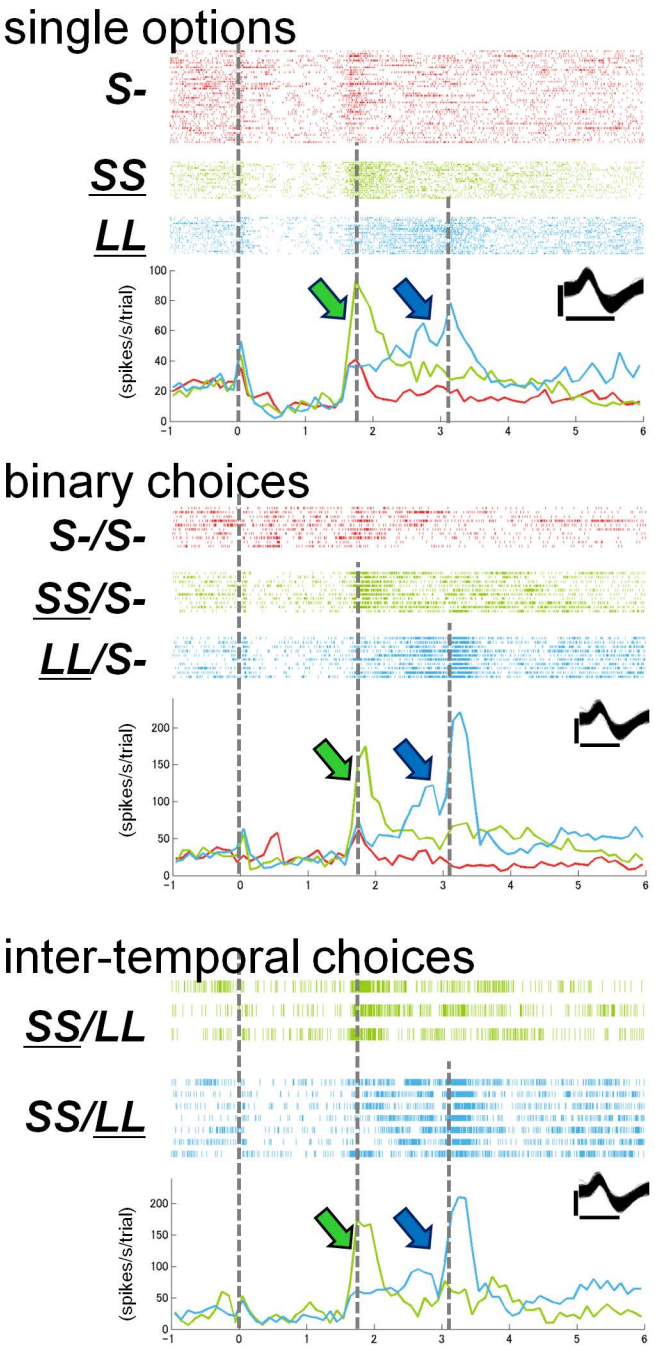

single options
S-
SS
LL
(spikes/s/trial)
binary choices
S-/S-
SS/S-
LL/S-
(spikes/s/trial)
inter-temporal choices
SS/LL
SS/LL
(spikes/s/trial)

Figure 4

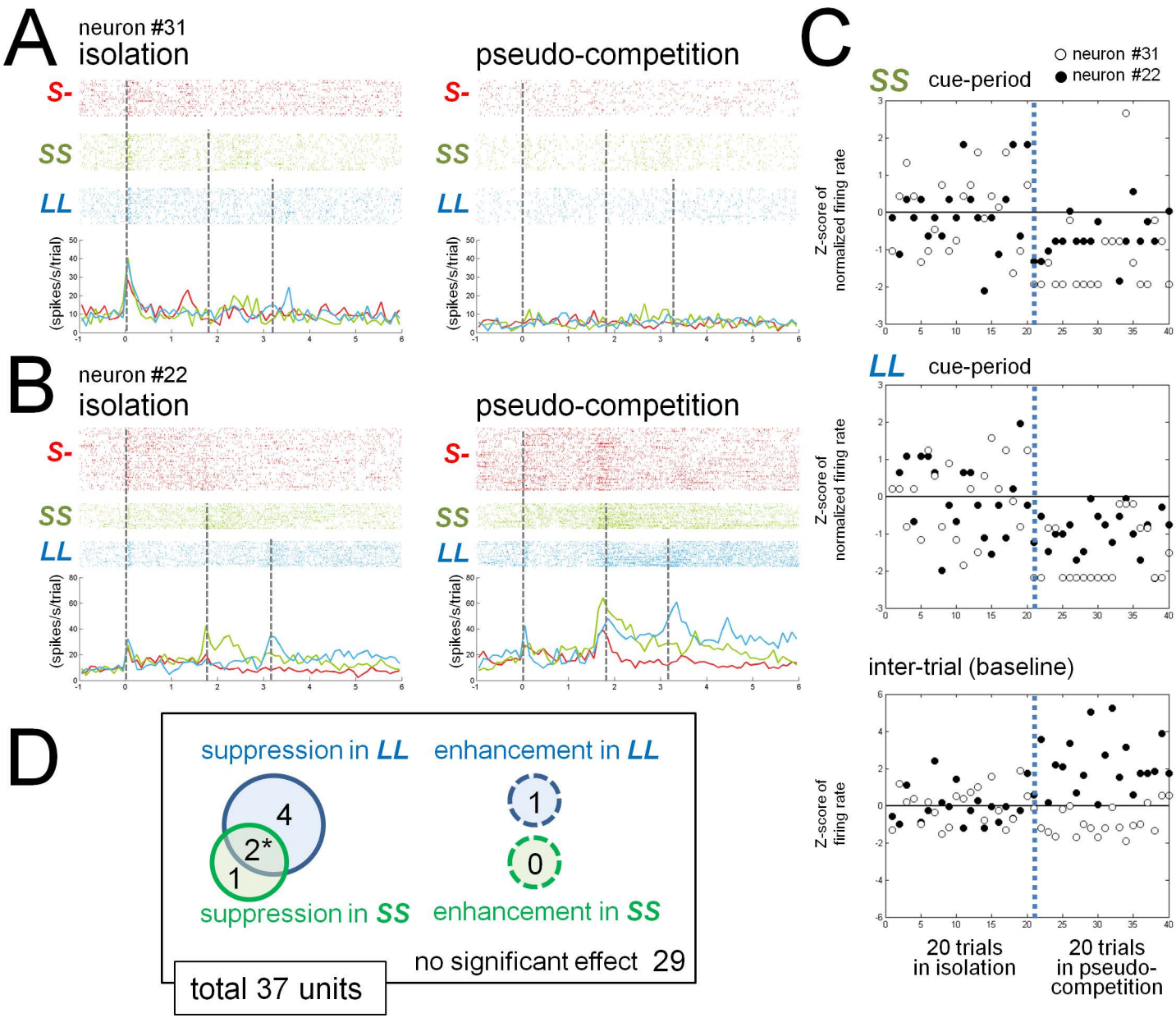

A
neuron #31
isolation
pseudo-competition
S-
SS
LL
(spikes/s/trial)
B
neuron #22
isolation
pseudo-competition
C
neuron #31
neuron #22
SS cue-period
LL cue-period
inter-trial (baseline)
Z-score of normalized firing rate
Z-score of firing rate
20 trials in isolation
20 trials in pseudo-competition
D
suppression in LL
enhancement in LL
4
2*
1
1
0
suppression in SS
enhancement in SS
no significant effect 29
total 37 units

Figure 5

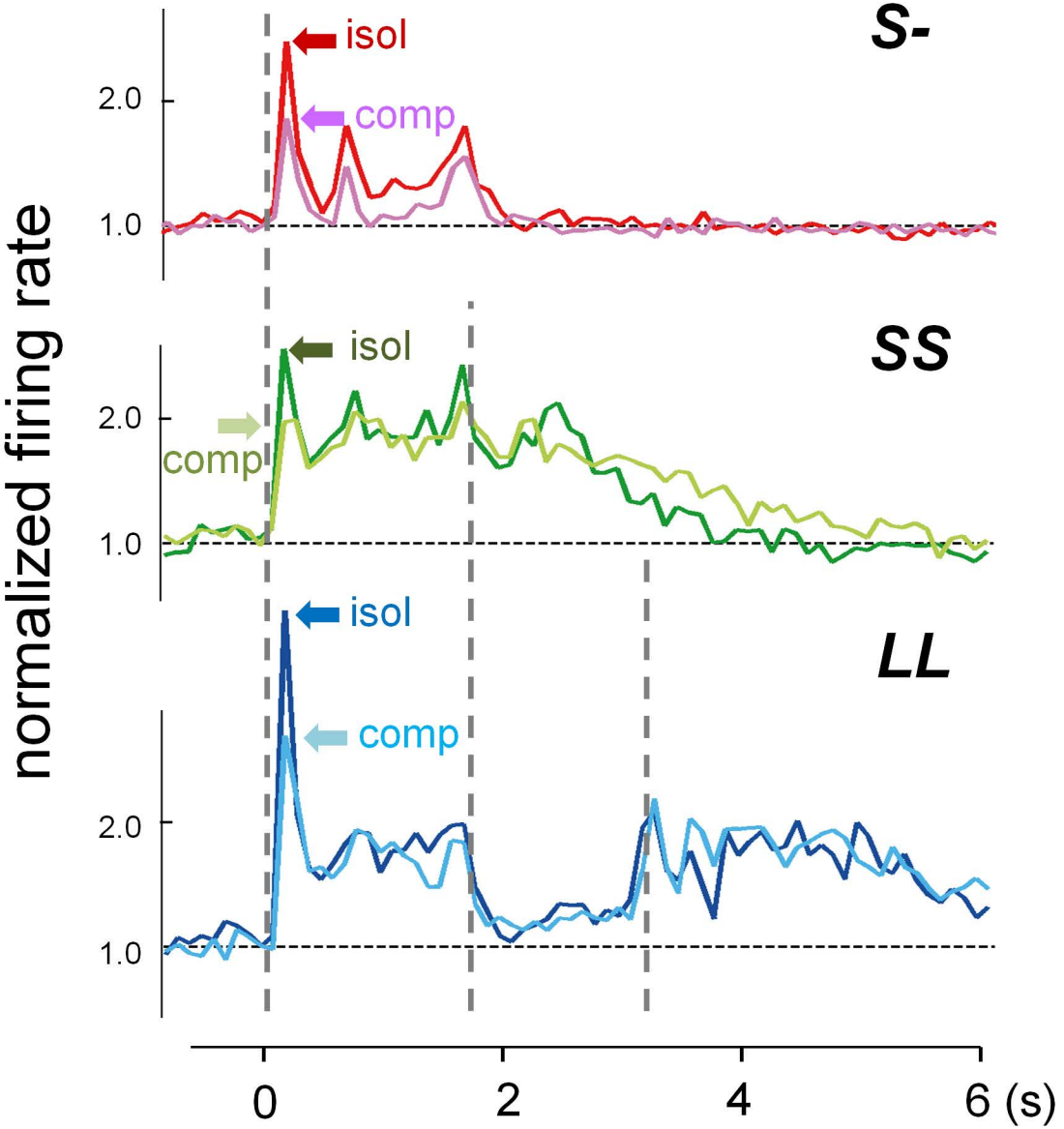

normalized firing rate
S-
isol
comp
2.0
1.0
SS
isol
comp
2.0
1.0
LL
isol
comp
2.0
1.0
0
2
4
6 (s)

Figure 6

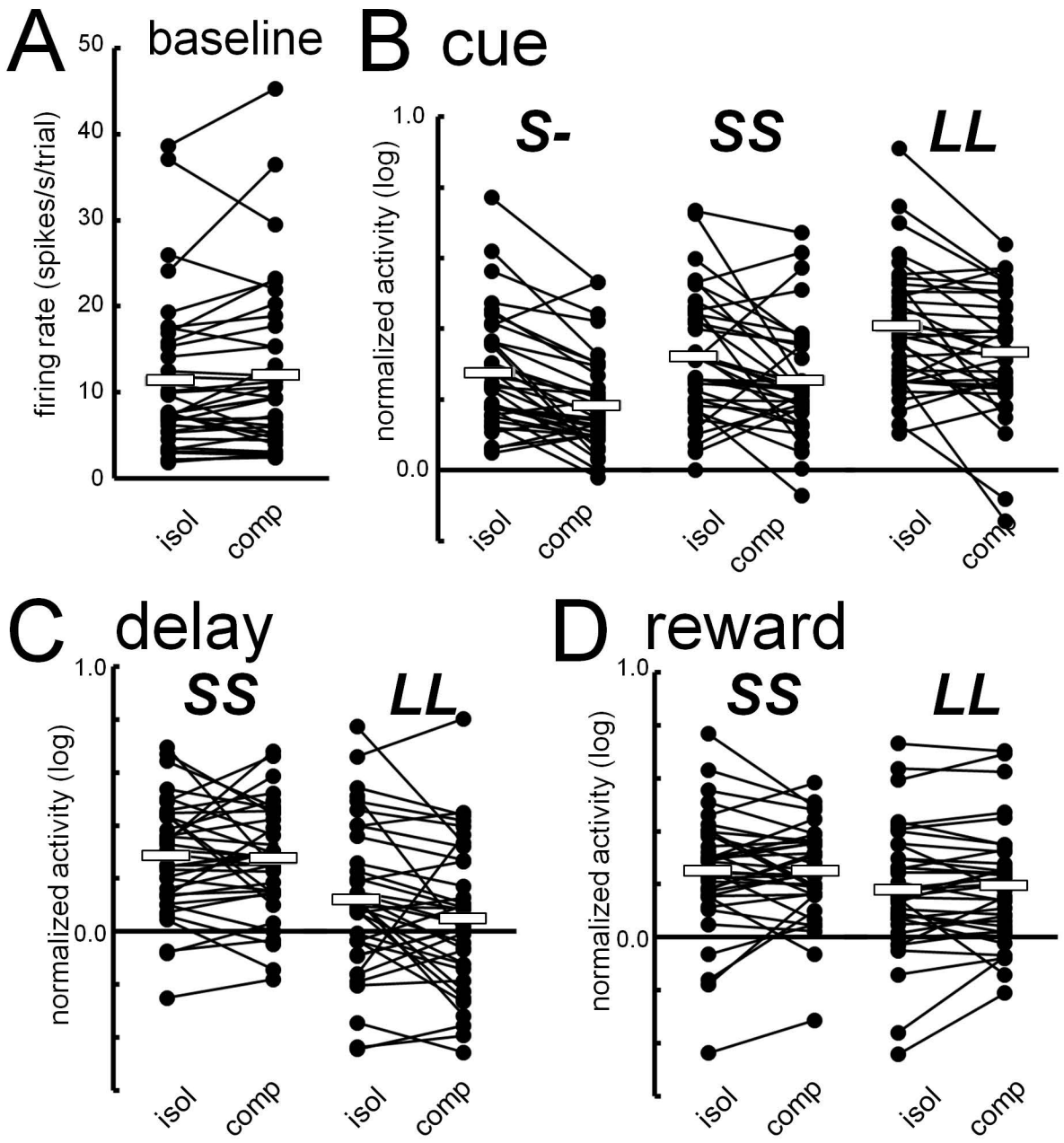

A
baseline
firing rate (spikes/s/trial)
50
40
30
20
10
0
isol
comp
B
cue
S-
SS
LL
normalized activity (log)
1.0
0.0
isol
comp
isol
comp
isol
comp
C
delay
SS
LL
normalized activity (log)
1.0
0.0
isol
comp
isol
comp
D
reward
SS
LL
normalized activity (log)
1.0
0.0
isol
comp
isol
comp

Figure 7

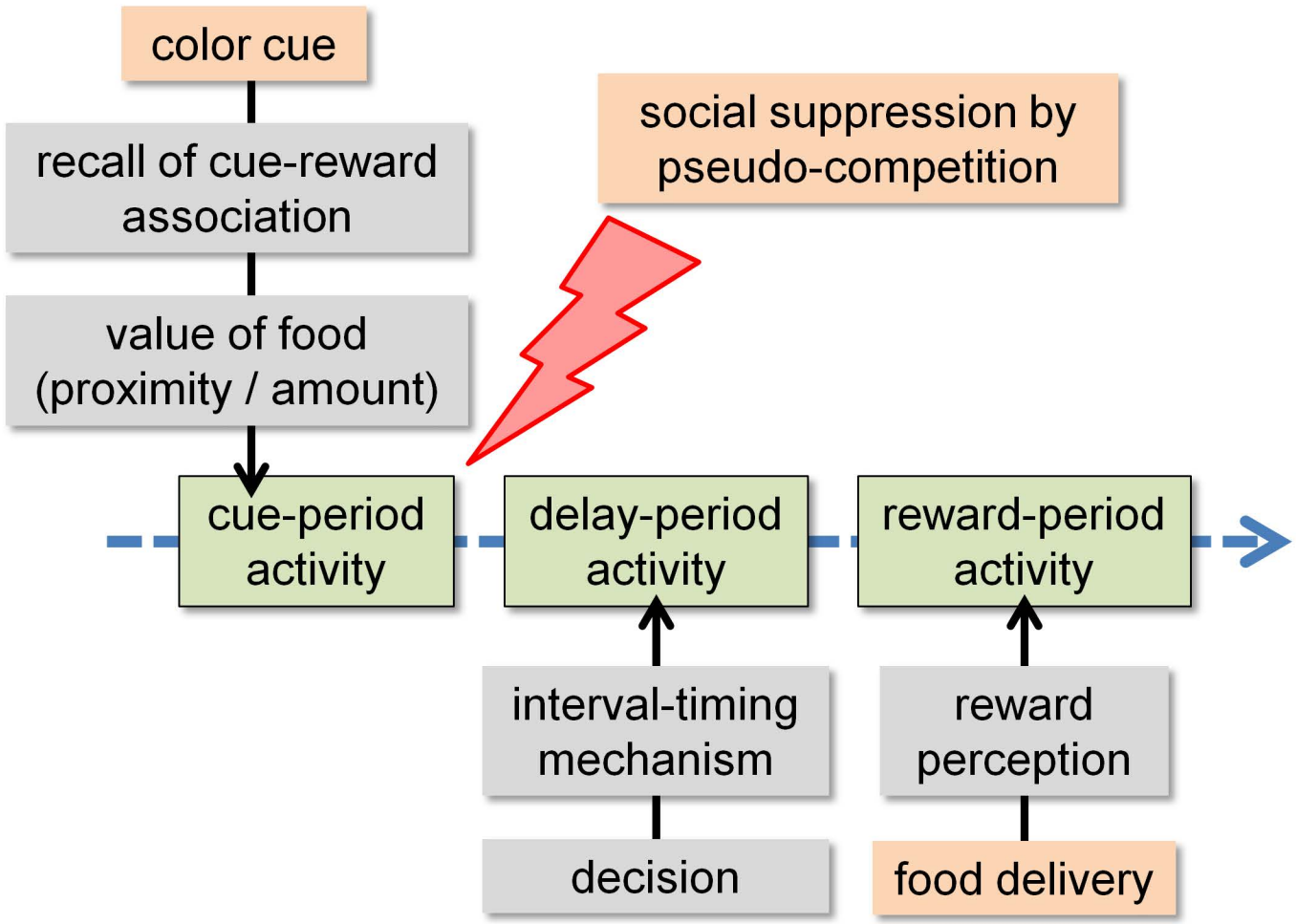

color cue
recall of cue-reward association
value of food (proximity / amount)
social suppression by pseudo-competition
cue-period activity
delay-period activity
reward-period activity
interval-timing mechanism
decision
reward perception
food delivery

Figure 8