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Title:

Dissociation of the neural substrates of foraging effort and its social facilitation in the domestic chick

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Abstract

The frequency or intensity of behavior is often facilitated by the presence of others. This social facilitation has been reported in a variety of animals, including birds and humans. Based on Zajonc's "drive theory," we hypothesized that facilitation and drive have shared neural mechanisms, and that dopaminergic projections from the midbrain to striatum are involved. As the ascending dopaminergic projections include the mesolimbic and nigrostriatal pathways, we targeted our lesions at the medial striatum (MSt) and substantia nigra (SN). We found that a bilateral electrolytic lesion of the MSt suppressed baseline foraging effort, but social facilitation was intact. Conversely, an electrolytic lesion targeted at the unilateral SN (on the right side) partially suppressed social facilitation, while baseline foraging effort remained unaffected. However, selective depletion of catecholaminergic (tyrosine hydroxylase immunoreactive) terminals by micro-infusion of 6-hydroxydopamine (6-OHDA) to bilateral MSt had no significant effects on foraging behavior, whereas it impaired formation of the association memory reinforced by water reward. Neurochemical assay by high-performance liquid chromatography also revealed a significant decrease in the dopamine and noradrenaline contents in MSt after 6-OHDA micro-infusion compared with intact control chicks. Thus, we conclude that the neural substrate of social facilitation can be dissociated from that responsible for reward-based foraging effort, and that ascending dopaminergic pathways do not appear to contribute to social facilitation. Based on our detailed analysis of the lesion areas, we discuss fiber tracts or neural components of the midbrain tegmental area that may be responsible for social facilitation.

Keywords: dopamine, drive, incentive motivation, ventral tegmental area, substantia nigra, ventral striatum, nucleus accumbens

Highlights:

- Electrolytic lesion of the medial striatum suppressed foraging effort.
- Electrolytic lesion of the lateral tegmentum suppressed social facilitation.
- Dopamine depletion in the medial striatum had no effect on foraging behavior.

1. Introduction

Social facilitation, a phenomenon originally described as an enhanced frequency or intensity of behavior in the presence of others [1,2], has been found in animals ranging from insects [3,4] to humans [5,6]. A variety of behaviors appear to be facilitated, i.e., motor activities, such as running [3], nest-building [4] and cycling [5], as well as cognitive tasks in humans (e.g., word association, [6]). Of these activities, foraging behaviors are socially facilitated in many vertebrates (e.g., fish [7], amphibians [8], birds [9,10], rats [11,12] and humans [12–14]), implying common psychological and neural processes.

As a generalizable account of social facilitation, Zajonc (1965) [15] proposed the “drive theory”. Specifically, he hypothesized that the presence of other individuals increases the general arousal or “drive” level, so that a dominant behavior (or well-learned behavior) is consequently facilitated. Here, Zajonc uses the term “drive” to denote a non-selective enhancer of behavior, just as Hull (1943) [16] argued in his classic study. In the 1960’s, the “drive” concept was challenged, and the field shifted its focus towards “incentive motivation” [17,18]. However, “incentive motivation” is not

mentioned in most recent studies of social facilitation. Note that the term “social facilitation” does not necessarily implicate any unitary mechanisms. Clayton (1978) [19] argued that the term should be used descriptively, and that the causal mechanisms could vary among different species and contexts.

Subsequently, based on human cognitive studies, Baron (1986) [20] developed “distraction-conflict theory” of social facilitation. He argued that the presence of others acts as a distractor, and attentional conflict occurs between an activity at hand and any other individuals present. He further noted the possibility that the conflict restricts the attentional focus of the subject, so that performance is facilitated. In concert with Baron’s theory, Huguet et al. (1999) [21] reported that performance on the Stroop task was socially facilitated. A meta-analysis of studies on human social facilitation ([22]) partially supported the Zajonc theory, although the analysis was generally in favor of the distraction-conflict theory. Baron (1986) [20] also argued that the distraction-conflict theory offered a parsimonious explanation for social facilitation in non-human animals. To the best of our knowledge, however, no empirical studies have tested the applicability of Baron’s theory to animal behavior.

Despite progress in the behavioral or cognitive studies, the neural basis of social facilitation has rarely been addressed. In a study using starlings, Cheng et al. (1999) [23] showed that the social facilitation of foraging behavior was reduced after lesions to the taeniae amygdala (TnA), an avian homologue of the mammalian amygdaloid complex. In the study, however, the authors analyzed behavioral synchronization rather than increases in the foraging effort, and so the observed lesion effects might be associated with other sociosexual behaviors, such as courtship [24] and copulation [25]. So far, the number of neurobiological study using animals is quite limited, likely

because an appropriate animal model has not been established.

The social facilitation of foraging behavior in the domestic chick [9,10] provides a unique opportunity to study the neural bases of this behavior because it is reproducible, thus allowing quantitative analyses [26]. Furthermore, the neural bases of foraging behavior have been well documented in a series of lesion [27–31] and electrophysiological experiments ([32–34]; also see Matsushima et al. [35,36] for reviews).

Based on Zajonc’s “drive” theory, we hypothesized that social facilitation and drive/incentive motivation have shared mechanisms. We were particularly interested in the role of dopaminergic projections from the midbrain to the striatum, as these could contribute to “drive” or incentive motivation [37–39]. In rats, both extensive dopamine depletion in the striatum [40] and systemic antagonism of dopamine transmission [41] are known to attenuate spontaneous foraging and bar pressing responses, respectively. In the avian brain, dopaminergic projections have been intensively studied [42–45], revealing two major ascending pathways that are conserved between birds and mammals [46].

The mesolimbic pathways from the ventral tegmental area (VTA) to the ventral striatum/nucleus accumbens play a critical role in controlling drive/incentive motivation [37,47]; but see [48]. The nigrostriatal projection is another major dopaminergic pathway that extends from the midbrain substantia nigra (SN) to the dorsal striatum, in which neurons represents expectation and delivery of food rewards [49–52]. Distinct functional roles have been suggested between these two pathways in mammals [53,54]. While the mesolimbic pathway is considered to be involved in the evaluation of and association between cues and outcomes [55,56], the nigrostriatal pathway is considered

to be critical for motor and action control [57,58].

There are two possible neural mechanisms underlying social facilitation. In one, social facilitation occurs through enhanced activity of the mesolimbic pathway, and the magnitude of a perceived food reward is augmented. In the other, social facilitation occurs through enhanced activity of the nigrostriatal pathway, and action-reward association is socially augmented. In this study, we wanted to examine whether these dopaminergic projections are required for social facilitation. Accordingly, we performed a series of lesion experiments in the medial striatum (MSt) and the SN using non-selective electrolytic lesions and dopamine-selective depletion by a localized infusion of 6-hydroxy dopamine (6-OHDA). Lesions placed in the MSt (or SN) were expected to impair the mesolimbic (or nigrostriatal) pathway, respectively; also see our supplementary material.

2. Materials and methods

2.1 Subjects

We obtained male domestic chicks (*Gallus domesticus*, White Leghorn strains) that were new hatchlings, i.e., post-hatch day 1 (presumed hatching day), from a local supplier (Iwamura Poultry Ltd./Hokkaido Central Poultry, Yubari, Japan). The chicks were paired and housed in transparent plastic cages (15 cm × 28 cm × 12 cm) under illumination from white LED lamps (12L: 12D; light period starting at 08:00) and thermo-controlled at about 28°C. We provided the chicks with two types of food: grains of millet and mash food. The total amount of food given per day was maintained such that (1) the body weight of the chicks gradually increased and (2) the chicks actively consumed food during the experiments. From post-hatch day 1, the chicks received 2 g

(post-hatch days 1–3) and 2.5 g (from day 4 onwards) of mash food. From post-hatch day 2, we also provided 2 g (days 2 and 3) and 2.5 g (from day 4 onwards) of grains of millet (per chick per day). Until day 2, all chicks were fed communally with their cage-mates. From day 3 onward, the chicks were fed solitarily in a cage that was visually separated by a black plastic wall, so that the chicks could not see their cage-mates eating food. With the exception of feeding time, the chicks were communally housed. Water was available *ad libitum*.

Experiments were conducted under the guidelines and approval of the Committee on Animal Experiments of Hokkaido University. The guidelines are based on the national regulations for animal welfare in Japan (Law for Humane Treatment and Management of Animals; after a partial amendment No. 68, 2005). After the experiments, the brains were dissected under deep anesthesia. In cases in which surgical operations were not conducted, the chicks were euthanized by carbon dioxide.

2.2 Apparatus

We used an I-shaped maze equipped with two parallel lanes (Fig. 1A-c, B-c; 12 cm × 88 cm × 40 cm high) that were separated by transparent Plexiglas. Terminal walls were painted red (left) or blue (right), and were equipped with a pair of terminal feeders that supplied food simultaneously in both lanes. The feeders supplied each lane with a single grain of millet. If not stated otherwise, the intervals between the food supply randomly varied from 10 to 20 sec (mean = 15 sec), and this schedule was referred to as VI15. Two sponge-covered food trays (3 cm × 4 cm × 2 cm deep) were placed adjacent to each other in the ends of the lanes. To prevent the chicks from associating the mechanical sounds generated by the feeders with the food reward, dummy motors driven at various intervals (uniformly distributed from 1.5 to 3.5 sec, mean = 2.5 sec)

were placed around the maze. The apparatus was placed in a dark room kept at *ca.* 25–30°C and illuminated under four 22-W white light bulbs. The feeders and dummy motors were controlled by microcomputers (Mindstorms RCX1.0, LEGO, Billund, Denmark).

We recorded the behavior of the chicks using a video camera (DCR-SR65, Sony Co., Tokyo, Japan; 30 frames per sec) for offline analysis. The video camera was positioned above the center of the I-maze, providing an aerial view of the subject and companion chicks. Each chick was individually marked with a small rectangular piece of fluorescent-colored tape (Yamato Co., Ltd., Tokyo, Japan) affixed to its head. The position of the fluorescent marker was traced via Move-Tr/2D 7.0 software (Library Co., Tokyo, Japan) and running distance was calculated based on the digitized trajectories of each chick.

2.3 Behavioral procedures

2.3.1 Habituation

At post-hatch days 4–7, the naïve chicks were habituated to the I-maze for 2 successive days. Paired cage-mates were placed at the midway point of the maze and given some food (*ca.* 100 grains of millet). After the chicks consumed the food, feeders started to deliver food according to the VI15 schedule. Two grains of millet were simultaneously supplied to the pair of chicks, so that each chick was expected to gain 1 grain.

2.3.2 Test

2.3.2.1 Effects of pairing on running distance

After the habituation, the chicks were subjected to a total of 8 tests: 4 tests on the 2 pre-operative days and 4 tests on the 2 post-operative days. The surgical procedure was

conducted on the day after the pre-operative tests, and the chicks were allowed to recover from the operation for 1–2 days before the post-operative tests.

Each test comprised an initial acclimatization phase and 3 subsequent phases: single #1, paired, and single #2 (Fig. 1A). During the acclimatization phase, the chicks foraged and consumed *ca.* 20 grains that were delivered to each feeder (40 grains in total) in advance. Then, after a short pause, the feeders started to supply food according to VI15 schedule. The single #1 phase consisted of 30 food deliveries (*i.e.*, 30 grains of millet) to both feeders, and usually lasted for *ca.* 8 min. The companion chick (cage-mate) was then introduced to the opposing lane of the maze, and the paired phase started, during which 30 grains were similarly delivered to the feeders in both lanes. Finally, the companion was removed from the maze and the subject resumed foraging in single condition (single #2 phase). After the food supply was terminated, the subject was left in the maze for an additional 2 min. In one test, each subjects gained 220 grains per individual in about 26 min.

2.3.2.2 Effects of changes in the feeding rate on running distance

After the final test was over, sham-operated chicks underwent a single foraging test that included 3 phases with different feeding rates (Fig. 1B). In the first and third phases, the feeding rate was set low, according to VI30 (variable intervals, mean = 30 sec) schedule, and the chicks received 15 food deliveries (*i.e.*, 15 grains) from each feeder in about 8 min. In the second phase, the feeding rate was set higher, according to VI10 schedule, and the chicks received 45 food deliveries (*i.e.*, 45 grains) from each feeder in about 8 min. The test was terminated 2 min after the subject consumed all of the delivered food.

2.3.3 Water–reinforced color discrimination task

After being deprived of water supply for 17-25 hrs, chicks were reinforced to selectively peck at a rewarding water bottle based on the plastic tape (yellow or green) wrapping around its outlet tube. One of the two bottles (colors) was associated with water (S+), and another bottle was just empty (S-), and the assignment was counter-balanced in each group of chicks. Note that the subject chicks had pecked at an unwrapped tube and gained water in their home cage. The behavioral task consisted of two sessions, a reinforcement (training) session and two probe (test) sessions. Chicks received the sessions in an experimental cage (15cm × 28cm × 12cm) equipped with two holes for water bottles.

A reinforcement session consisted of 2-8 blocks. In one block, chick was presented with a water bottle (S+ color) twice and then with an empty bottle (S- color) twice at one side of the cage, and we counted the number of pecks in these 4 trials, each for 1 min at interval >30 sec. We repeated the reinforcement up to 8 consecutive blocks alternately at two sides of the cage, until the chick actively responded to the S+ color with > 9 pecks during the 1-min trials at both sides. As a behavioral measure, we recorded total number of pecks in each trial.

In probe sessions, chick was tested in binary choice of colors. Two bottles (yellow and green, both empty) were simultaneously presented twice for 1 min each (at interval >30 sec) with its side counter-balanced. Two probe sessions were given; the first session on the day of reinforcement, and the second session on the next day. In the probe sessions, we counted the number of pecks at yellow and green outlet tubes.

2.3.4 Statistical tests

The behavioral data were tested according to non-parametric methods (Wilcoxon-Mann-Whitney test, Friedman test and Kruskal–Wallis test) using R (version 3.0.2,

Windows 64-bit version) [59] and the additional packages (RcmdrPlugin.EZR version 1.20 [60], exactRankTests version 0.8-28 and coin version 1.0-24 [61]).

2.4 Surgery

The chicks were anesthetized by intramuscular injection of about 0.4ml ketamine-xylazine cocktail (a 1:1 mixture of 10 mg/ml ketamine [Daichi Sankyo Co. Ltd., Tokyo, Japan] and 2 mg/ml xylazine [Sigma–Aldrich Co. LLC, St. Louis, MO, USA]).

Supplementary injections (0.10 ml) were given to maintain stable anesthesia. The chicks were then fixed to a stereotaxic apparatus (SR-5N, Narishige, Tokyo, Japan). The skin over the skull surface was incised, and the dura mater was cut to expose the brain.

To produce electrolytic lesions, we used sharp steel electrodes made from an insect pin (type #00; Shiga Konchu, Tokyo, Japan) that had been coated with enamel paint (Tamiya Inc., Shizuoka, Japan), except for a 0.5 mm at the tip. For the MSt lesion, the electrode was vertically inserted into the brain at the following coordinates: anterior–posterior 3.3–4.0 mm anterior from bregma, 1.0 mm lateral from the midline, and 5.9–6.5 mm deep from the brain surface. For the SN lesion, we tilted the electrode anteriorly by 14°. The coordinates were 0.8 mm anterior to 0.2 mm posterior from bregma, 2.0 mm lateral from the midline, and 8.3–8.8 mm deep from the brain surface. In some cases, chicks received two lesions per side. Constant current pulses with alternating polarity (amplitude = ± 1.5 –1.8 mA, pulse duration = 50 ms, repetition rate = 10 Hz, lasting for 1 min) were delivered by an electric stimulator (SEN-3301 and isolating unit SS-403J, Nihon Kohden, Tokyo, Japan) to the inserted electrode. A silver wire reference electrode was placed on the caudal skull.

To selectively lesion catecholaminergic neurons and terminals, we infused 6-hydroxydopamine hydrobromide (6-OHDA, Sigma–Aldrich; 5 or 10 mg/ml saline with

0.2% ascorbic acid) by a 1- μ l Hamilton microsyringe (#80100) and a motor-driven injector (IMS-10, Narishige). Chicks received 1 or 2 injections per site, in which 0.6 μ l was infused slowly over a period of *ca.* 13 min. Thus, a total of 3–6 μ g (6-OHDA groups) or 12 μ g (high-dose 6-OHDA group) was infused. After the infusion was completed, the syringe was left in the brain for an additional 5 min to ensure diffusion of the infused drug. For the MSt lesion, we adopted following coordinates: 3.3–4.0 mm anterior from bregma, 1.0 mm lateral from midline, 5.9–6.2 mm deep from brain surface. For SN lesion, we tilted the syringe penetration anteriorly by 14°. The coordinates were 0.4mm anterior to 0.3 mm posterior from bregma, 1.5–2.0 mm lateral from midline, 8.2–8.5 mm deep from brain surface.

For the sham operation, we either inserted an electrode without applying current or gave an injection of 0.6 μ l ascorbic acid solution (vehicle) into either the MSt or SN in a total of 11 chicks.

The incised skull was covered with a gelatin sponge (Spongel®, Astellas Pharma Inc., Tokyo, Japan), and the skin flap was fixed using superglue (Aron Alpha®, Toagosei Co. Ltd., Tokyo, Japan). The post-surgery chicks were housed singly overnight for recovery, and were thereafter housed with their cage-mates.

2.5 Histology

Two days after the final test (*i.e.*, the post-operative 4th test), chicks were deeply anesthetized by an intra-muscular injection of *ca.* 0.8ml ketamine-xylazine cocktail, and transcardially perfused with a fixative (4% paraformaldehyde in 0.1 M PB, pH = 7.4). In chicks with electrolytic lesions, the brain tissue (telencephalon and midbrain) was embedded in yolk and post-fixed in the same fixative for >2 days at 4°C. The tissue was then cut into 100 μ m frontal sections using a vibrating microslicer (DTK-1000, Dosaka

EM, Kyoto, Japan). The sections were mounted and stained with cresyl violet.

In chicks with 6-OHDA lesions, we examined the brain tissue by tyrosine hydroxylase (TH) immunohistochemistry. These chicks were similarly perfused and the dissected brain tissue was post-fixed for 6 hrs at 4 °C, and then in 20% sucrose in PBS at 4 °C for overnight. The brain tissue was frozen at -80 °C for storage, and cut into 40 µm frontal sections by a freezing microtome (Yamato Kohki Industrial Co. Ltd., Saitama, Japan). Floating sections were then stained with antibody (anti-TH rabbit polyclonal antibody, diluted ×1,000; Merck Millipore Co. Billerica, MA, USA), and with secondary antibody (biotinylated goat anti-rabbit IgG; diluted ×300; Vectastain® Elite® ABC kit, PK-6100, Vector Laboratories, Inc., Burlingame, CA, USA). The reaction product was visualized with DAB (in: DAB Peroxidase Substrate Kit; SK-4100, Vector Laboratories), and the sections were mounted, dehydrated, and cleared.

We followed the stereotaxic atlas of the chick brain by Kuenzel and Masson (1988) [61]. Further abbreviations of neural nuclei and nuclei boundaries followed Bálint et al. (2011) [62] and the report on nomenclature reform [45].

2.6 Neurochemical assays

In some chicks, we assayed contents of monoamines and their metabolites in striatum (MSt and lateral striatum including globus pallidus); nidopallium was also analyzed as a control region. After the behavioral examinations, whole brain was dissected out under a deep anesthesia, and frozen in dry ice and stored at -80°C. Rostral part of the brain was trimmed, and frontal plane of the telencephalon was exposed at the level of *ca.* A10.0-A9.4 on a sliding microtome with a freezing unit (-20°C). Neural tissue was manually punched out by using a stainless-steel tubing (i.d. 1.0 mm, o.d. 1.2 mm) with an inner rod, homogenized by ultra-sonification in 0.2 M perchloric acid, and

centrifuged (11,000 g for 15 min, 4°C). The punch-out tissues were merged from bilateral hemispheres, and the remaining brain tissue was photographed and stored in a fixative.

The supernatant and pellet was assayed as follows; the procedure was basically in accordance with Izumi *et al.* (2012) [62]. The supernatant was promptly mixed with 20% 1 M sodium acetate, and following monoamines were assayed by high-performance liquid chromatography (HPLC); dopamine (DA), noradrenaline (NA), serotonin (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). The HPLC system was composed of chromatopump (EP-300), reverse-phase ODC column (SC-50DS) and electrochemical detector (ECD-300, Eicom Co. Kyoto, Japan). The mobile phase (0.5 ml/min) was 0.04 M sodium acetate-citrate acid buffer containing 17% methanol, 190 mg/l sodium 1-octanesulfonate and 5 mg/l EDTA disodium. Protein content in the pellet was assayed by Bradford's method [63] using a spectrophotometer (GeneQuant 100, General Electric Co. Fairfield, CT, USA).

3. Results

We conducted surgeries and I-maze testing on a total of 62 chicks. Of these, 3 chicks were discarded after the operation: 2 chicks with MSt lesions (one with an electrolytic lesion and one with a 6-OHDA lesion) that stopped foraging both in the home cage and the I-maze, and 1 chick with an SN electrolytic lesion that exhibited biased pecking in the silicon clay test (see below in Fig. 3). The following results are based on data from the remaining 59 chicks, which showed spontaneous foraging and maintained normal postures of the head and body.

In another set of 13 chicks, we conducted water-reinforced color discrimination task and neurochemical assay; 6 chicks received micro-infusion of 3 $\mu\text{g}/\text{site}$ 6-OHDA in bilateral MSt, and the other 7 un-operated chicks served as intact control. Furthermore, in a separate set of 10 chicks, we conducted 6-OHDA infusions targeted at the VTA. We found that the TH immunoreactivity in the VTA was suppressed in 5 out of the 10 chicks. However, in these 5 chicks with suppressed TH immunoreactivity in the VTA, the SN was also damaged (see Supplementary material, Fig.S1). As we were unable to selectively lesion the mesolimbic pathway in this manner, we decided to make lesions in the MSt as an alternative.

3.1 Effects of pairing and increase in feeding rate on running distance

In accordance with our previous report [26], the running distance of the subjects increased as soon as the companion chick was introduced. Fig. 1A shows a representative trial (Fig. 1Aa) together with the population data (Ab). Each set of connected symbols represents an individual chick, and the data obtained in the 4 tests (over 2 days) were averaged and plotted. The Friedman test revealed a significant difference among the 3 phases ($\chi^2 = 18.7273$, $df = 2$, $p = 0.0001$; $n = 11$ sham-operated chicks). Post-hoc pairwise comparisons conducted using the Wilcoxon signed rank test with the Holm correction revealed a significant difference between the single phase #1 and the paired phase ($p = 0.0029$), as well as between the single phase # 2 and the paired phase ($p = 0.0029$), but we found no significant difference between the single phases #1 and #2 ($p = 0.1020$). Thus, our data indicates that the pairing facilitated running distance both immediately and reversibly.

(Figure 1 around here)

Running distance also increased as soon as the feeding rate increased (Fig. 1B), but

this increase was less distinct compared with the pairing effect described above. At the population level, the Friedman test revealed a significant difference among the three phases ($\chi^2 = 13.5556$, $df = 2$, $p = 0.0011$; $n = 9$ chicks; the remaining 2 chicks were not tested). Post-hoc pairwise comparisons conducted using the Wilcoxon signed rank test with the Holm correction revealed a significant difference between the VI10 phase and each of the two VI30 phases ($p = 0.0120$), but we found no significant difference between the two VI30 phases ($p = 0.7340$).

3.2 Behavioral effects of lesions

We used the following two variables as behavioral indices: (1) running distance in the single phases as an index of the foraging effort, and (2) increase in running distance in the paired phase as an index of social facilitation. The increase (Δ) in running distance was given as follows:

$$\overline{single} = \frac{single\ #1 + single\ #2}{2}$$

$$\Delta\ in\ running\ distance = paired - \overline{single}$$

3.2.1 Effects of MSt and SN lesions on running distance

We compared the running distance in the single phases ($\overline{single}$) among the control group (sham-operated chicks, $n = 11$) and the 5 groups of lesioned chicks, namely, MSt electrolytic ($n = 8$), MSt 6-OHDA ($n = 8$), SN electrolytic ($n = 13$), SN high-dose 6-OHDA ($n = 6$), and SN 6-OHDA ($n = 13$). As shown in Fig. 2, we averaged and plotted the data ($\overline{single}$ and Δ) obtained in the 4 consecutive tests (conducted over 2 days) for each individual. For details about the histological examinations, see below (Figs. 5–7). In the pre-operative tests (Fig. 2A), the Kruskal–Wallis test revealed a significant difference among the 6 groups ($\chi^2 = 11.9311$, $df = 5$, $p = 0.0357$). However, post-hoc Steel's multiple comparisons failed to reveal significant differences in $\overline{single}$ between

the sham group and the all 5 lesion groups. In the post-operative tests (Fig. 2C), we found that chicks with the MSt electrolytic lesion had a lower $\overline{single}$. The Kruskal–Wallis test revealed a significant difference in $\overline{single}$ among the groups ($\chi^2 = 23.9774$, $df = 5$, $p = 0.0002$), and subsequent post-hoc Steel's multiple comparisons revealed a significant difference between running distance in chicks with the MSt electrolytic lesion ($p = 0.0018$) compared with the sham controls.

(Figure 2 around here)

We attempted a bilateral electrolytic lesion of the SN, but found that the surgical operation severely diminished spontaneous food/water pecking. Spontaneous locomotor activities were also reduced, and the chicks did not forage or eat millet in either the I-maze or the home cage. These chicks subsequently exhibited a reduced body weight, so we immediately terminated the experiment in this group. In contrast, the chicks with unilateral lesions of the SN showed selective suppression of social facilitation.

3.2.2 Effects of MSt and SN lesions on social facilitation

We compared Δ in running distance among the sham controls and the 5 groups of lesioned chicks. In the pre-operative tests, the Kruskal–Wallis test revealed no significant differences among the groups (Fig. 2B; $\chi^2 = 7.9871$, $df = 5$, $p = 0.1570$). In the post-operative tests (Fig. 2D), we found that chicks with the SN electrolytic lesion and chicks with the SN high-dose 6-OHDA lesion had a lower Δ , compared with the sham controls. The Kruskal–Wallis test revealed a significant difference in the Δ value among the 6 groups ($\chi^2 = 29.6290$, $df = 5$, $p < 0.0001$). Subsequent Steel's multiple comparison tests revealed significant differences in electrolytic lesion ($p = 0.0014$) and in high-dose 6-OHDA lesion ($p = 0.0118$) compared with the sham controls.

After the behavioral tests were complete, we further tested all 59 chicks for normal

visuomotor coordination while pecking at millet grains. Fig. 3 shows two representative example chicks, one sham-operated chick (a, b) and another with an electrolytic lesion to the right SN (c, d). The chicks were given a transparent plate of soft silicon clay on which 12 grains of millet were placed. As each chick pecked at the grains, they left beak marks on the clay surface. We found that the marks were localized in close vicinity to the grains in all groups.

3.2.3 Effects of MSt 6-OHDA lesion on water-reinforced color discrimination task and monoamine contents in the striatum

To examine how severely the dopaminergic terminals were lesioned after 6-OHDA micro-infusion to MSt, we conducted another behavioral and tissue assay using chicks which were age-matched to the subjects examined for the foraging behavior. The post-infusion survival period before the behavioral examination (3 days) and brain sampling for the tissue assay (7 days) was also matched.

(Figure 4 around here)

In the reinforcement session, one of the cue colors (yellow and green) was associated with water reward (S+) and the other with empty bottle (S-). The chicks with MSt 6-OHDA lesion ($n = 6$) were compared with the intact chicks ($n = 7$) in their behavior (Fig. 4Aa,b). Ratios of S+ pecks (pecks in the S+ trials / sum of pecks in both S+ and S- trials) were not significantly different between the groups (Wilcoxon-Mann-Whitney rank sum test; $W = 11.5$, $p = 0.1929$). Similarly, the total number of pecks did not differ ($W = 30$, $p = 0.2343$). The number of blocks also did not significantly differ (intact: 3.71 ± 0.84 blocks, 6-OHDA: 2.83 ± 0.31 blocks, $W = 24$, $p = 0.6795$).

In the probe sessions, bottles were empty, and chicks were not rewarded in the binary choice trials even when S+ was pecked. In the first probe session performed at 1-

1.5 hrs after the reinforcement session, most chicks in both groups were inactive and the total number of pecks fell below 50 in 11 out of 13 chicks; we therefore did not include the first probe data. In the second probe session performed at 21-27 hrs after the reinforcement (Fig. 4Ac,d), on the other hand, the lesion chicks showed a significantly lower choice ratio than the intact control ($W = 39, p = 0.0082$), whereas chicks showed considerable number of pecks (> 50 in all 13 chicks) without significant difference between the groups ($W = 22, p = 0.9452$).

After 6-OHDA micro-infusion, neurochemical assay revealed selective decrease in the contents of dopamine and noradrenaline, but not other monoamines and metabolites (Fig. 4Ba,b). In the MSt, both dopamine (DA, $W = 42, p = 0.0012$) and noradrenaline (NA, $W = 40, p = 0.0047$) were significantly lower in the 6-OHDA chicks than the intact control, whereas no significant difference was found in DOPAC, HVA, 5-HT and 5-HIAA. The sampled areas in the MSt (Fig. 4C) matched well with those that showed suppressed TH-immunoreactivity (see below in Fig. 5Ad, Bd₃). In the LSt/GP (Fig. 4Bb), no significant difference was found in any monoamines examined. In the nidopallium, the contents was lower than 8 pg/μg protein in all subjects (not shown).

3.3. Histological examination of lesions

Detailed histological examinations revealed that the tissue damage that accompanied the SN electrolytic lesion and the SN high-dose 6-OHDA lesion could be responsible for the observed suppression in social facilitation. Selective damage to dopaminergic neurons did not appear to cause behavioral effects in either the MSt or SN 6-OHDA lesion groups.

3.3.1 Tissue damage and suppressed TH immunoreactivity in the MSt lesion groups

As described above, the MSt electrolytic lesion reliably induced bilateral tissue damage. However, we found considerable inter-individual variance; thus, it is possible that the extent of behavioral change could be ascribed to different sizes or locations of the lesion. Based on the *single* values obtained by the sham-operated chicks (mean = 115.8 m, unbiased variance = 203.6 m², n = 11), we estimated the 95% confidence range to be 87.8–143.7 m. Of the 8 chicks in the MSt electrolytic group, 7 chicks fell below the estimated 95% range and were thus categorized as having exhibited a large behavioral effect. As shown in Fig. 5Ab, we superimposed the tissue damage in these 7 chicks, while the remaining 1 chick that exhibited no behavioral effect is shown in Fig. 5Ac. Representative Nissl stained micrographs are shown in Figs. 5Bb and 5Bc. In the 7 chicks with large effect, the MSt, NAcc, BSTl, and VP were almost completely destroyed. In the remaining 1 chick, on the other hand, lesion was much smaller.

(Figure 5 around here)

We observed considerable suppression of TH immunoreactivity in the MSt 6-OHDA lesion group (Fig. 5Ad); compare a representative micrograph of the lesion (Bd₃) with a sham-operated control (Ba₃). Note also that Nissl-stained sections did not reveal tissue damage; compare the lesion (Fig. 5Bd_{1,d2}) and control (5Ba_{1,a2}). We therefore assume that catecholaminergic terminals were selectively ablated without non-specific damages in the MSt.

3.3.2 Tissue damage and suppressed TH immunoreactivity in the SN lesion groups

The SN electrolytic lesion reliably induced unilateral tissue damage in the right midbrain tegmental areas, although we observed considerable inter-individual variance. Similarly to the, we estimated the 95% confidence range for social facilitation to be 56.7–98.7 m based on the Δ in running distance exhibited by the sham-operated chicks

(mean = 77.7 m, variance = 115.1 m², n = 11). Of the 13 chicks that received the SN electrolytic lesion, 10 fell below this range and were thus categorized as exhibiting a large effect. We considered the remaining 3 chicks to have shown no effect. We superimposed the regions with tissue damage for animals in these sub-groups (Fig. 6Ab and Ac). In the 10 chicks who exhibited a large behavioral effect, the lateral and dorso-lateral areas of the tegmentum were completely destroyed, including the SNc and SNr together with the PPN and tracts such as the tractus occipitomesencephalicus (OM) and ansa lenticularis (AL). Conversely, in the remaining 3 chicks that showed no behavioral effect, the lesion was smaller.

(Figures 6 and 7 around here)

The 6 operated chicks who received a SN high-dose 6-OHDA lesion also had marked unilateral tissue damage (Fig. 7Aa & Ab; also see photomicrographs in Ca & Cb). These chicks were categorized into two subgroups, one with a large behavioral effect with respect to Δ in running distance (n = 4) and another with no behavioral effect (n = 2). Importantly, the lesioned regions overlapped in both of these sub-groups, and the lesions were smaller than those in the SN electrolytic lesion groups (Fig. 6). In contrast, chicks in the SN 6-OHDA lesion group (n = 13) showed little to no tissue damage (Fig. 7Ac). In fact, we observed a small amount of tissue damage in only 1 out of the 13 chicks. When observed at a higher magnification, the Nissl stained sections revealed a clear loss of large-sized neurons in the SN area (Cc₁ and Cd₁ for the lesion side, and Cc₂ and Cd₂ for the intact side), suggesting that the cell bodies of dopaminergic neurons were selectively lost. TH-immunostaining failed to detect marked differences among the groups of chicks with 6-OHDA lesions (Fig. 7B).

4. Discussions

Based on the present results, we conclude that the neural substrates of foraging effort and its social facilitation can be dissociated. Bilateral electrolytic lesions targeted at the MSt (MSt electrolytic lesion; Fig. 5) suppressed the *single*, which was used as a measure of reward-based foraging effort (Fig. 2C). Conversely, a unilateral lesion to the right tegmental area, including the SN (SN electrolytic lesion and SN high-dose 6-OHDA lesion; Fig. 6 and 7) suppressed social facilitation (Fig. 2D), whereas the *single* was unchanged. In the following, we will discuss the possible neural systems involved in these behaviors.

4.1. Neural mechanisms of foraging effort

4.1.1. Dopaminergic / noradrenergic fiber terminals in the MSt

We had initially supposed that dopaminergic projections to the striatum are critical for foraging effort. This was because incentive motivation appears to be modulated by dopamine in goal-directed behavior in which efforts are exerted towards a specific goal or reward [63,64]. In accordance with this idea, effort aversion has been linked with dopamine depletion. For instance, when rats with surgically lesioned dopamine neurons were required to choose between a preferred food option (with lever press; high-effort) and less-preferred food (without any effort), they chose the latter option [65,66] (but see [67]).

In the present study, on the other hand, micro-infusion of 6-OHDA to bilateral MSt had no effect on baseline foraging effort (Fig. 2C). Another behavioral measure (the number of pecks in the reinforcement session) was also unimpaired (Fig. 4Ab). However, the 6-OHDA micro-infusion effectively suppressed formation of association memory (Fig. 4Ac) without impairing pecking vigor (4Ad). The present results are in

good concert with our previous study in which excitotoxic lesions to bilateral MSt caused an anterograde (but not retrograde) amnesia in water-reinforcement color discrimination task [Izawa, E.-I., Zachar, G., Aoki, N., Koga, K., Matsushima, T. (2004), Lesions of the vento-medial basal ganglia impair the reinforcement but not the recall of memorized color discrimination in domestic chicks. *Behavioural Brain Research*, **136**: 405-414]. Involvement of the dopamine transmission in MSt in the memory formation has already been suggested in behavioral study using the one-trial passive avoidance learning [Kabai, P., Stewart, M. G., Tarcali, J. & Csillag, A. (2004), Inhibiting effect of D1, but not D2 antagonist administered to the striatum on retention of passive avoidance in the chick. *Neurobiology of learning and memory* **81**: 155–8], and also in the activity-dependent synaptic plasticity [Matsushima, T., Izawa, E.-I., and Yanagihara, S. (2001), D1-receptor dependent synaptic potentiation in the basal ganglia of quail chicks, *NeuroReport* **12**: 2831-2837].

Further neurochemical assay (Fig. 4B) and TH-immunostaining (Fig. 5) indicate apparent decrease in catecholamines selectively in MSt. We assume that catecholaminergic terminals were selectively ablated without nonspecific damages, because (1) contents of monoamines other than catecholamines (namely, 5-HT and 5-HIAA) did not differ (Fig. 4Ba), and (2) Nissl staining revealed no apparent tissue damage after 6-OHDA micro-infusion (Fig. 5Bd₃).

The extent of dopamine depletion in our study (Fig. 4B; 39.8% of the control) was comparable to those found in previous study using rats [65], in which dopamine suppression as strong as 38.7% of the control resulted in significant effort aversion. Stronger depletion might impair foraging effort, however, our pilot experiments with 3-10 times higher dose of 6-OHDA (9-30 µg/site) always caused a nonspecific tissue

damages similar to the electrolytic lesion (data not shown).

We assume also that noradrenaline (NA) may not play a significant role in foraging effort. NA-ergic neurons are generally supposed to be involved in arousal and attention in both mammals and birds [68,69], and they are also lesioned by 6-OHDA infusions [70]. In domestic chicks, NA-immunoreactive fibers are densely distributed in the NAc and medial part of the lobus parolfactorius (LPO; [71]). After the nomenclature reform [45], the LPO was replaced by a set of nuclei in the ventromedial striatum, namely the MSt, the NAc, and the ventral pallidum [62]. We expected that a considerable number of NA terminals would be ablated in the chicks with the MSt 6-OHDA lesion (Fig. 5Ad) in our study. However, we noticed no associated behavioral effects (Fig. 2C, D).

The discrepancy between our findings and mammalian studies might be explained by different species of animals studied. Otherwise, it is due to different experimental paradigm we adopted in our study. While instrumental action (lever pressing: [65], climbing a barrier: [66]) was necessary for rats to obtain a preferred food, the chicks in our study simply had to approach the food tray. Salamone and Correa (2012) [72] suggested that dopamine in the ventral striatum contributes specifically to instrumental or conditioned behavior, and not consummatory or unconditioned behavior.

Taken together, the selective suppression of catecholamines in MSt, at least at the level sufficient for memory impairment, is ineffective on the foraging efforts. Other pharmacological methods than 6-OHDA micro-infusion must be applied in future studies.

4.1.2. Other neural components in the MSt

Electrolytic lesions to the MSt resulted in a suppressed foraging effort (Fig. 2C),

suggesting that efferent neurons in the MSt might play a role in the execution of foraging behavior. In our previous studies, populations of neurons in the MSt (and surrounding areas including the NAc core) responded to food rewards or visual color cues associated with food [32–34]. Tract-tracing studies [62,73,74] have revealed that neurons in the MSt and NAc project to the VTA, SNc, and midbrain reticular formation. However, unilateral lesions of these midbrain tegmental nuclei did not suppress the *single* (Fig. 2C). It is possible that MSt neurons regulate motor output indirectly through their projections to the lateral striatum and regions further downstream [73]. Furthermore, neurotensinergic pathway from NAc and parabrachial nucleus is suggested to be involved in feeding processes [Bálint, E., Balázs, T., Zachar, G., Mezey, S. & Csillag, A. (2014) Neurotensin: revealing a novel neuromodulator circuit in the nucleus accumbens–parabrachial nucleus projection of the domestic chick. *Brain Structure and Function*.]. The pathways specifically involved in the foraging effort remain elusive.

4.2. Neural mechanisms of social facilitation

4.2.1. Dopaminergic / noradrenergic neurons in the tegmentum

We assume that nigrostriatal dopaminergic projections do not play a critical role in social facilitation, because a micro-infusion of a moderate dose 6-OHDA to the SN produced no behavioral effects (Fig. 2D). NA-ergic neurons are distributed in the area spanning from the locus ceruleus (LoC) to the nucleus subcoeruleus ventralis (SCv) [71], but these are located much more caudally, and thus were expected to be intact in the present study. As the chicks with high-dose 6-OHDA SN lesions showed suppressed social facilitation, this outcome is to be ascribed to nonspecific tissue damage (Fig. 7A and C). Similar nonspecific tissue damage has been reported to occur at high doses of 6-OHDA [75].

That the extensive unilateral damages to the SN and surrounding tegmental tissues did not cause sensorimotor deficits, nor abnormal posture in the chicks, was unexpected. In rats, however, an injection of 6-OHDA to the unilateral SN induces asymmetric posture and rotational behavior [76]. Abnormal postures and body tremor have also been reported in adult pigeons after electrolytic or chemical lesions to the unilateral SN and adjacent areas [77]. This difference might be due to the age of the experimental subjects, as motor deficits induced by intracranial 6-OHDA are smaller in neonatal compared with adult rats [78]. It is important to note that the post-operative recovery period was set at two months in the rat study [78], and it was only two days in our study using chicks. It is unlikely that post-lesion compensatory changes would have occurred during such a short recovery period.

In this study, as argued above, we applied the highest dose of 6-OHDA to induce depletion of catecholamines without nonspecific tissue damage. Yet, we are unable to reject a possibility that a higher dose of 6-OHDA could be applied, considering that the MSt 6-OHDA chicks developed a weak preference with the ratio >0.5 in the test session (Fig. 4Ac). We need other pharmacological methods than 6-OHDA micro-infusion to examine the functional roles of catecholamines on social facilitation.

4.2.2. Other components in the tegmentum

We concluded that the midbrain tegmentum, particularly the lateral and dorsolateral areas including the SN, is critical in mediating the social facilitation of foraging effort. Because of the large size of tissue damage found in the chicks with the SN electrolytic lesion (Fig. 6Ab), we were unable to identify the neuronal populations that were specifically responsible for the observed suppression of social facilitation. Non-dopaminergic tegmental neurons around the SN might be a candidate, as these may

directly and locally control foraging behavior without the involvement of descending signals from the telencephalon. Interestingly, birds are still capable of pecking at food objects [79] and approaching moving objects [80] after decerebration. Indeed, Sowards and Sowards (2003) [81] proposed that animals have innate processes for visual cognition of conspecifics, and that such processes are entirely mediated by sub-telencephalic structures. In the following, we will consider the potential role of GABA-ergic neurons in the SNr and cholinergic neurons in the PPN in social facilitation.

4.2.2.1 GABA-ergic neurons

As in mammals, the avian SN is composed of two sub-regions, namely the SNc and SNr (Fig. 6Aa). These sub-regions are contiguous with each other, but have different cytoarchitecture [45]. While the SNc is characterized by densely packed dopaminergic neurons [46], the SNr contains GABA-ergic projection neurons and dopaminergic cell bodies [82,83]. Considering that the chicks who received SN 6-OHDA infusions exhibited no behavioral changes (Fig. 2D), it is possible that the suppressed social facilitation observed following the electrolytic lesion was caused by a loss of GABA-ergic neurons in the SNr. This interpretation is not consistent with data from mammal studies. As the SNr is thought to exert motor control through its GABA-ergic neurons [84–87], functional loss of these is expected to enhance motor activities [88]. However, lesions in the present study did not increase foraging effort.

4.2.2.2 Cholinergic neurons

The pedunculopontine nucleus (PPN) is a tegmental nuclei located ventrally to the SNc. In birds, the PPN is thought to be the major source of cholinergic innervations in the midbrain, diencephalon, and telencephalon [89]. The suppressed social facilitation observed in the present study might be due to reduced cholinergic activity. Indeed, the

PPN is included in the area of tissue damage in chicks with SN electrolytic and high-dose 6-OHDA lesions (Figs. 6, 7). On the other hand, Nissl-stained sections revealed intact PPN neurons in chicks with moderate-dose 6-OHDA infusions to the SN (data not shown), in which social facilitation was comparable to the sham controls. The PPN might thus be involved in social facilitation.

Several features of the PPN fit well with this idea. In birds, the PPN is known to receive projections directly from the ventral pallidum [90]. In mammals, in addition to receiving projections from the ventral pallidum [91], the PPN has reciprocal connections between the superior colliculus, SN, subthalamic nucleus, and reticular formation [91,92]. From the viewpoint of anatomical resemblance, the avian PPN is thought to be a homolog of the mammalian PPN [45]. However, to the best of our knowledge, the avian PPN has not been systematically investigated in terms of function and neuronal connectivity. Functionally, the mammalian PPN is known to be involved in locomotion [86,93,94], general attention [95], wakefulness (or arousal) [96,97], and possibly in behavioral control based on reward [98, 99]. As social facilitation is thought to be induced by elevated arousal [15] or attentional conflict [20], the PPN might be responsible for social facilitation.

4.2.3 Fiber tracts descending from the telencephalon

Descending efferent fibers from telencephalic structures may contribute to social facilitation, as the lesioned areas in our study included some of these tracts. Following the 3 major descending pathways (Fig. 8), we will consider the role of the tractus septomesencephalicus (TSM), OM, and AL.

(Figure 8 around here)

The TSM originates from the hyperpallium apicale and projects to the optic nucleus

of the thalamus, nucleus geniculatus lateralis pars ventralis, and optic tectum [100]. The TSM is thought to play a role in visuomotor control [101]. In our study, the TSM can be disregarded, as it remained intact in most of the chicks with SN electrolytic lesions (Fig. 5A). Thus, we focused on the other two descending pathways.

The OM originates from the arcopallium and projects to the lateral (parvocellular) reticular formation [102]. The OM is thought to control pecking behavior in a lateralized manner. Unilateral left and bilateral OM lesions suppress response key pecking, while lesions in the unilateral right OM have no effect [103]. Descending projections from the right arcopallium via the OM might be lateralized to social stimuli in the context of foraging behavior. However, bilateral excitotoxic lesions to the ventral arcopallium in chicks caused work cost aversion in terms of food pecking, but failed to impair behavioral execution [30,31]. Thus, the arcopallium is a strong candidate for social facilitation.

The AL includes descending fibers from the globus pallidus (GP), leading to the anterior/posterior nucleus of the ansa lenticularis (ALa and ALp, respectively) and the SN [91]. The ALa and ALp are considered to be the avian homologs of the mammalian subthalamic nucleus, and even a unilateral and partial lesion of the ALa induces nonspecific motor deficits [104]. In preliminary trials, we found that unilateral lesions to the GP revealed similar motor deficiencies, such as asymmetric posture and locomotion. However, we have no available evidence regarding the involvement of the AL in social facilitation.

4.2.4. Amygdala

The TnA is an avian homologue of the mammalian amygdaloid complex [23, 45], making it an additional candidate. Though most TnA efferent fibers project to the

medial septum and the hypothalamus, some descend to the midbrain through the OM. In a study using starlings, TnA lesions were found to reduce “social facilitation” of foraging behavior in terms of reduced behavioral synchronization [23]. In the present study, however, we did not examine TnA and its efferents.

4.2.5. Functional lateralization

In this section, we consider functional [105] and neuroanatomical [106] lateralization. In domestic chicks, conspecific recognition appears to be lateralized in the right hemisphere (i.e., the left visual hemifield) and foraging appears to be lateralized in the left hemisphere (the right hemifield) [107]. In the present study, we systematically searched right tegmental areas in view of this reported lateralization, and found that social facilitation was suppressed following lesion (but only partially). However, preliminary experiments also revealed a comparable suppression of social facilitation after electrolytic lesion targeted at the left tegmentum (unpublished observation). Future studies should address functional lateralization, and focus on identifying the neuronal components involved in social facilitation.

4.3. Is Zajonc’s “drive” theory appropriate?

The social facilitation of foraging effort in chicks did not appear to involve dopaminergic pathways. If these pathways are related to “drive”, then we are unable to accept Zajonc’s theory as a way to account for social facilitation in chicks. Alternatively, it might be too simplistic to assume that “drive” is functionally linked to dopamine system.

The present results do not provide us with any conclusive ideas as to the validity of Baron’s (1986) [20] “distraction-conflict theory”, as we did not examine whether companion chicks could induce attentional conflict. It is important to note that Baron’s

(1986) [20] theory is compatible with Zajonc (1965)'s theory. Baron (1986) [20] suggested that attentional conflict initiated by the presence of other individuals could elevate "drive" as well as restrict attentional focus. As Clayton (1978) [19] clearly pointed out, the mechanisms underlying socially facilitated behavior vary among different species, and we thus we should carefully investigate the neural substrates in each case.

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Figure legends

Fig. 1. Behavioral paradigm for measuring the foraging effort and social facilitation. Chicks ran back and forth between two feeders at each end in an I-shaped maze, and the trajectory of movement was video recorded and analyzed off-line. (A) Social facilitation by pairing. (a) Examples of running trajectory. The lower trajectory indicates the movement of the subject chick, and the upper one indicates that of the companion. One test trial consisted of 3 phases (single #1, paired, and single #2), in each of which chicks (both subject and companion) gained an equal amount of food (30 grains) from each of

the red and blue feeders in about 8 min. Chicks thus gained 180 grains in one trial. (b) Running distance in each phase (m) is plotted ($n = 11$ chicks) as connected symbols for each chick. (c) A grain of millet was delivered according to VI15 schedule (variable intervals with average = 15 sec) at each feeder without any sensory cues. Inset figure shows a sample food delivery pattern. (B) Facilitation of running distance by an increase in the rate of food supply. Chicks were tested in isolation. (a) An example of the running trajectory of a subject chick. A consecutive series of VI30 (variable interval around the average of 30 sec), VI10, and VI30 phases (each for about 8 min). (b) Running distance was similarly plotted ($n = 9$ chicks). (c) In the VI30 and VI10 phase, both feeders delivered single grains according to VI30 and VI10 schedules, respectively. *, $P < 0.05$; **, $P < 0.01$.

Fig. 2. Running distance (measured in isolation; A,C) and social facilitation (Δ increase in running distance during pairing; B,D) in the sham group and 5 groups of lesioned chicks. A and B show the pre-operative data, while C and D show the post-operative data. The median (horizontal bars) and upper- and lower-quartiles (boxes) are shown. Whiskers indicate the most extreme data point (< 1.5 times the interquartile range), and open circles indicate outliers. *, $P < 0.05$; **, $P < 0.01$.

Fig. 3. Millet grains placed on a transparent plate of soft silicon clay (a, c) and marks made by an individual pecking subject (b, d). Photos a and b show the marks made by a sham-operated chick (#130613B), while c and d show those made by a chick with a unilateral electrolytic lesion in the right tegmentum (#130613KN) that showed suppressed social facilitation. Note that the sensori-motor coordination of pecking

behavior remained intact.

Fig. 4. (A) Effects of 6-OHDA MSt lesion in water-reinforced color discrimination task. (a) Ratio of S+ pecks (pecks in the S+ trials / sum of pecks in both S+ and S- trials) and (b) total number of pecks in the reinforcement session. (c) Ratio of S+ pecks in unreinforced binary choice trials (pecks at the S+ color tube / sum of pecks at both S+ and S- tubes) and (d) total number of pecks recorded in the second probe session. Mean $\pm$ SEM are shown. (B) Contents of monoamines and their metabolites (pg/ μ g protein) measured in MSt (a) and LSt/GP (b) after MSt 6-OHDA lesion; * and ** indicates significance at $p < 0.05$ and $p < 0.01$, respectively. DA: dopamine, NA: noradrenaline, 5-HT: serotonin, DOPAC: 3,4-dihydroxyphenylacetic acid, HVA: homovanillic acid, and 5-HIAA: 5-hydroxyindoleacetic acid. (C) Sampled areas in the striatum superimposed on a frontal section at the level of A9.4.

Fig. 5. (A) Histological reconstruction of the MSt lesions. Lesioned areas are superimposed on frontal sections of the telencephalon. (a) Schematic illustration of TH-immunoreactive fiber terminals (shaded areas) in the intact brain. (b and c) Chicks with electrolytic lesions were categorized into two groups according to the *single* during the post-operation tests; i.e., those with a large effect (b; $n = 7$) and those with no effect (c; $n = 1$). For chicks with 6-OHDA lesions (d), striatal areas with suppressed TH-immunoreactivity are similarly superimposed. (B) Photomicrographs of Nissl-stained sections at a low magnification (a_1 , b, c and d_1), high magnification (a_2 and d_2) and TH-immuno-stained sections at a low magnification (a_3 and d_3); (a_1 - a_3), sham; (b,c), MSt electrolytic lesion; (d_1 - d_3), MSt 6-OHDA lesion. BSTl: lateral part of the bed nucleus of

the stria terminalis, E: entopallium, FPL: fasciculus prosencephali lateralis, GP: globus pallidus, HA: hyperpallium apicale, LPS: pallio-subpallial lamina, LSt: lateral striatum, M: mesopallium, MSt: medial striatum, N: nidopallium, NAcr: rostral pole of the nucleus accumbens, NAcc: nucleus accumbens core, NAcs: nucleus accumbens shell, TO: olfactory tubercle, TSM: tractus septopallio- mesencephalicus, VL: lateral ventricle, VP: ventral pallidum.

Fig. 6. (A) Histological reconstruction of tissue damage in chicks with unilateral electrolytic lesions targeted at the SN. (a) Schematic illustration of TH-positive cell bodies (dots). (b and c) Lesioned chicks were categorized according to the Δ in running distance during the post-operation tests. (B) Representative micrographs of Nissl-stained sections from a sham control chick (a) and a chick with an electrolytic-lesion with a large effect (b). (B) Photomicrographs of Nissl-stained sections from a (a) sham and (b) electrolytically lesioned chick with a large effect. A11d: A11 dopamine cells dorsal part, EW: nucleus of Edinger-Westphal, ExM: external mammillary nucleus, IF: interfascicular nucleus, IPC: interpeduncular nucleus, caudal subnucleus, IsMc: nucleus isthmi pars magnocellularis, IsPc: nucleus isthmi pars parvocellularis, ME: median eminence, MLd: nucleus mesencephalicus lateralis, pars dorsalis, MM: medial mammillary nucleus, medial part, mPAG: mesencephalic periaqueductal gray, NIII: nervus oculomotorius, OMN: oculomotor nucleus, p3Tg: P3 tegmentum, PPN: pedunculopontine tegmental nucleus, RM: retromammillary area, SNc: substantia nigra, compact part, SNr: substantia nigra, reticular part, SpM: nucleus spiriformis medialis, VTA: ventral tegmental area.

Fig. 7. (A) Histological reconstruction of tissue damage in chicks with unilateral 6-OHDA lesions targeted at the SN. Lesioned chicks were categorized according to the Δ in running distance during the post-operation test (a: large effect; b: no effect). All chicks with a lower dose of 6-OHDA (c) were categorized as showing no effect. (B) Suppressed TH immunoreactivity was compared among these 3 groups by superimposing opaque white plates. Note the comparable loss of TH-immunoreactive cell bodies in all three groups of chicks. (C) Photomicrographs of TH-immuno-stained sections at a low magnification (a, b, c1 and c2) and Nissl-stained sections at a high magnification (d1 and d2). A high dose of 6-OHDA produced distinct tissue damage (a and b), and a low dose caused suppressed TH immunoreactivity (c1) compared with the contralateral side (c2). At a higher magnification (d1 and d2), revealed that neurons with larger cell bodies (possibly DAergic neurons in SN) had been selectively diminished.

Fig. 8. Schematic illustration of three major descending pathways from the telencephalon to midbrain. In A4.0 and A3.4, TH-positive cell bodies (Fig. 6Aa) and tissue damages with large effect (6Ab) are superimposed on the pathways from the arcopallium (OM), and the GP (AL). See the text for further discussions.

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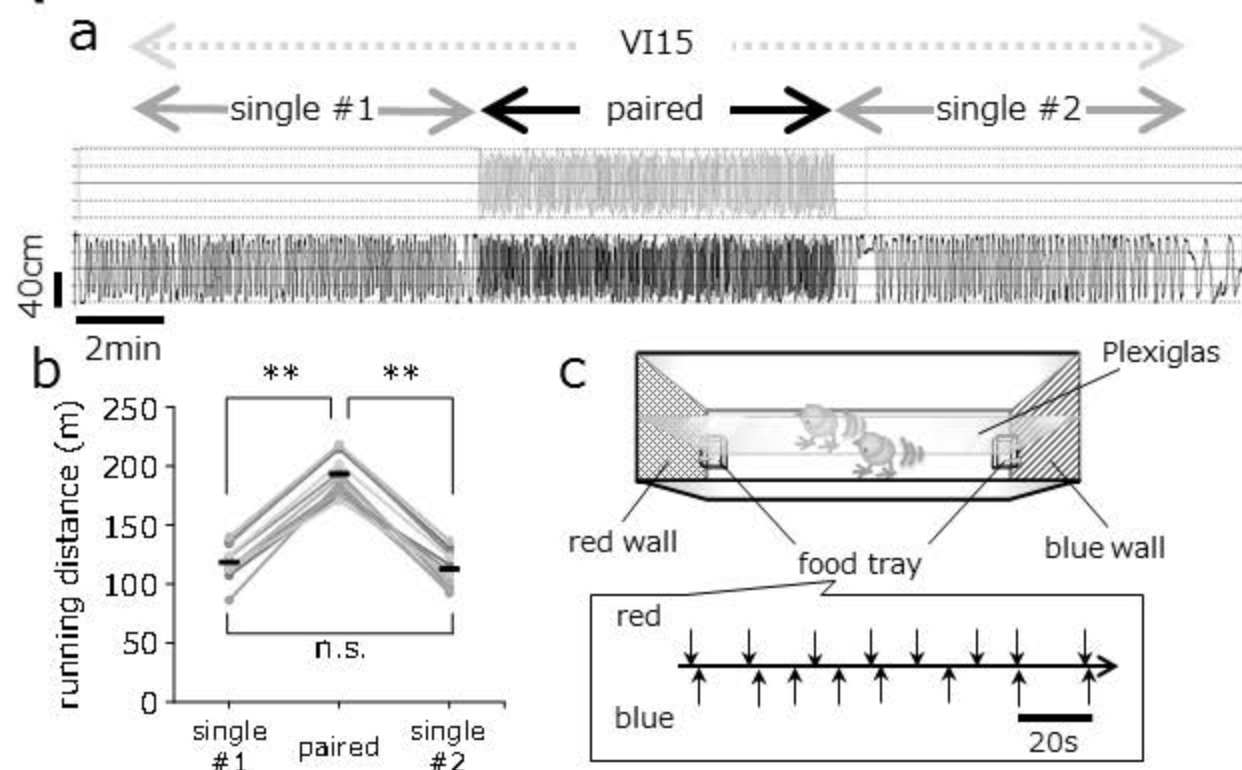
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Figure 1

A



B

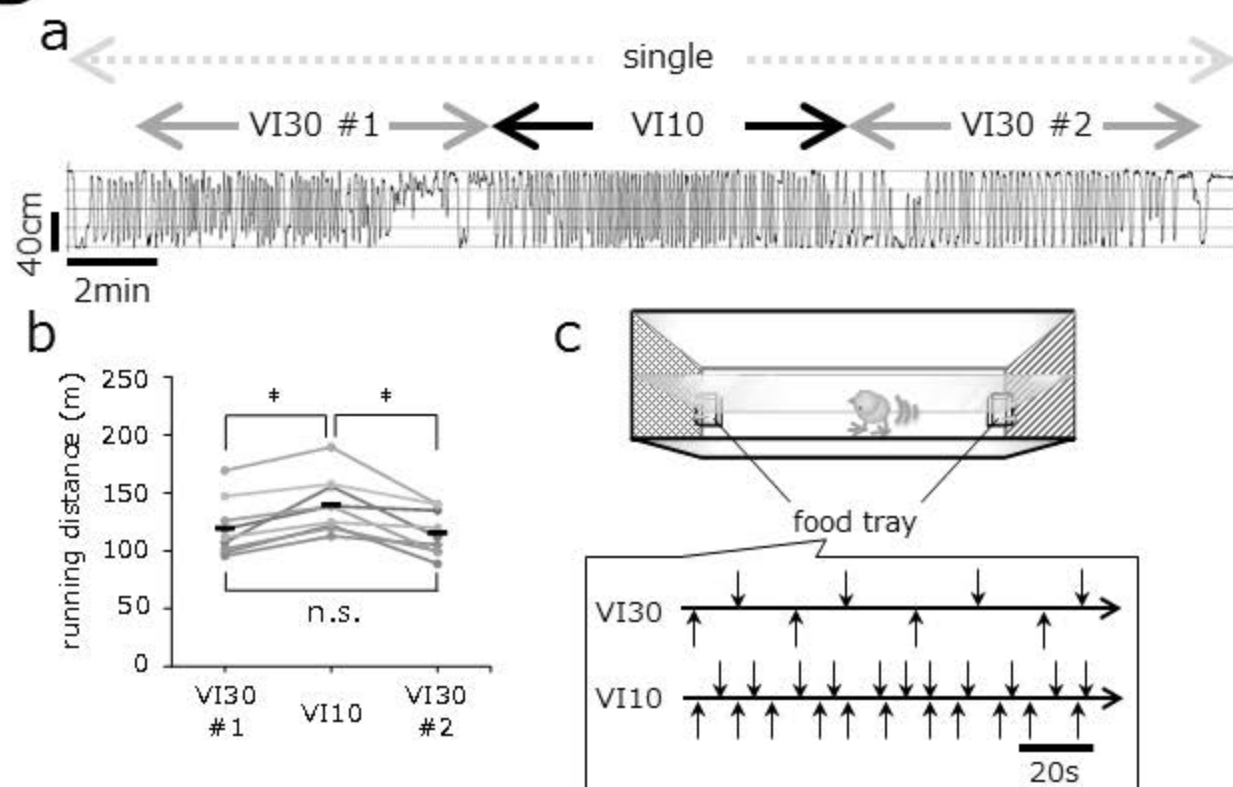


Figure 2

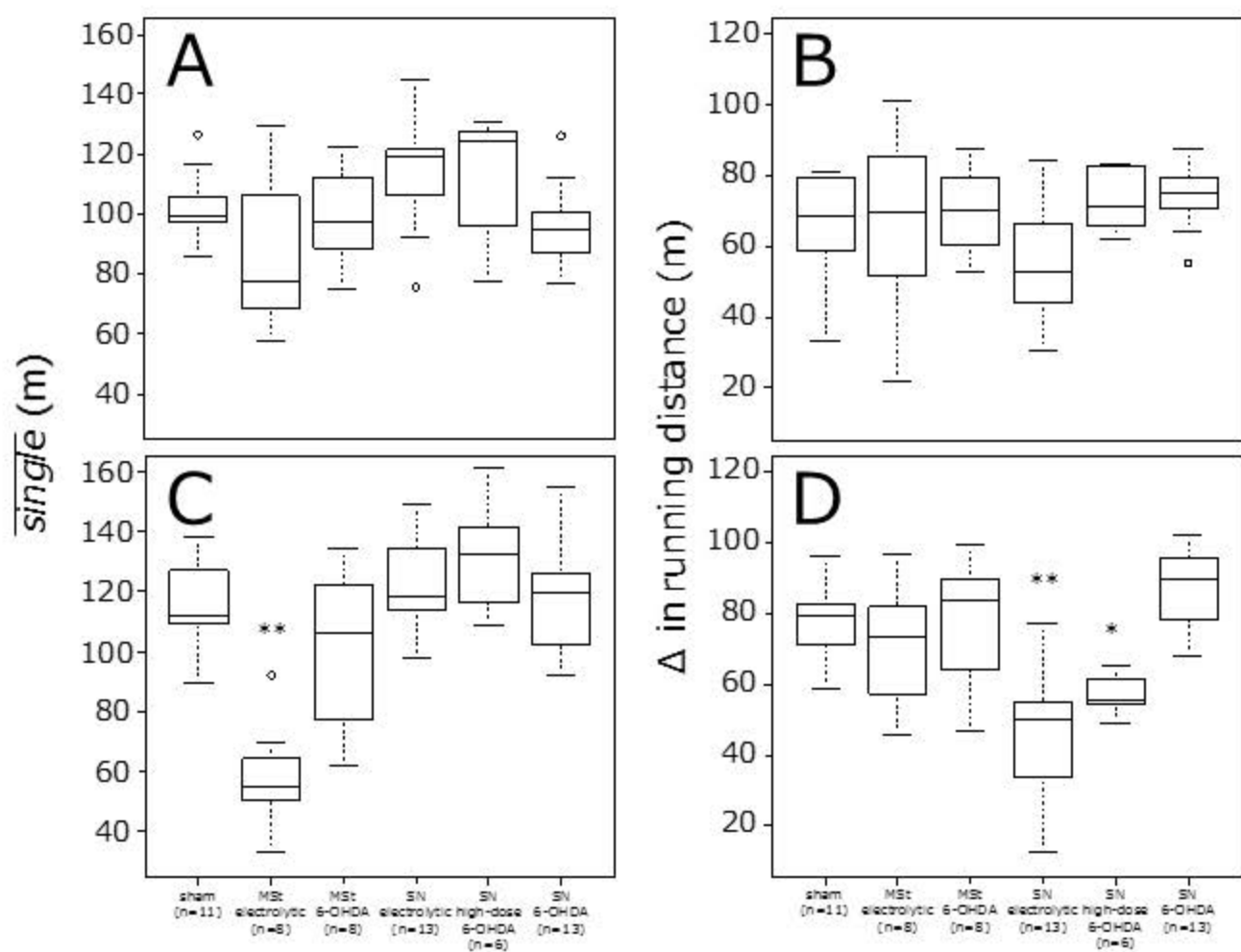
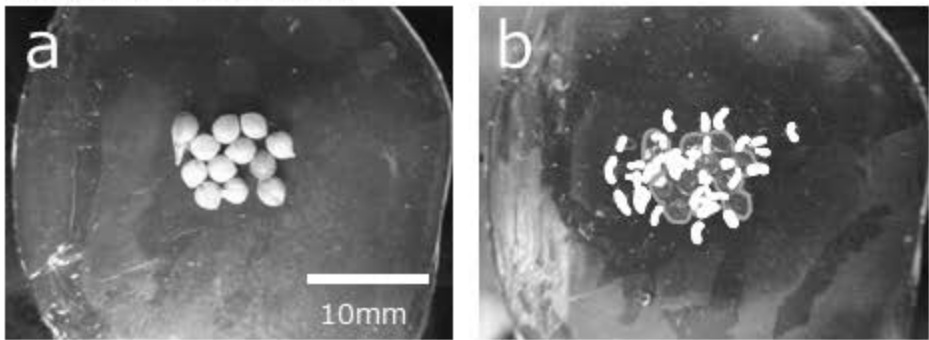


Figure 3

sham (#130613B)



lesion (#130613KN: unilateral right SN, electric lesion)

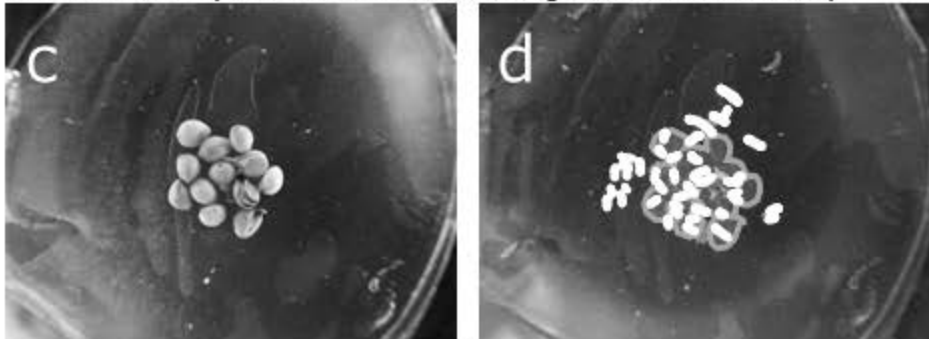


Figure 4

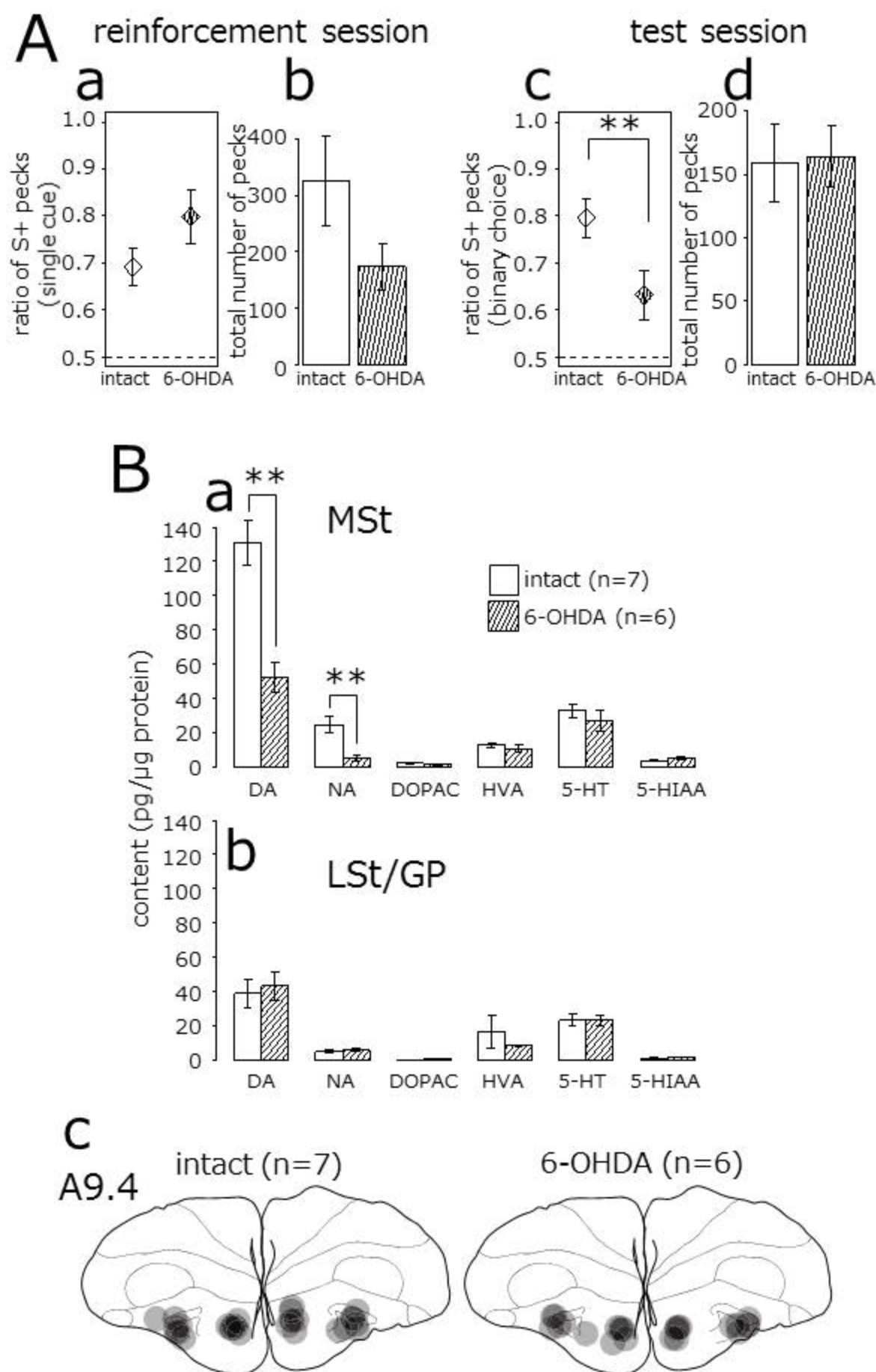


Figure 6

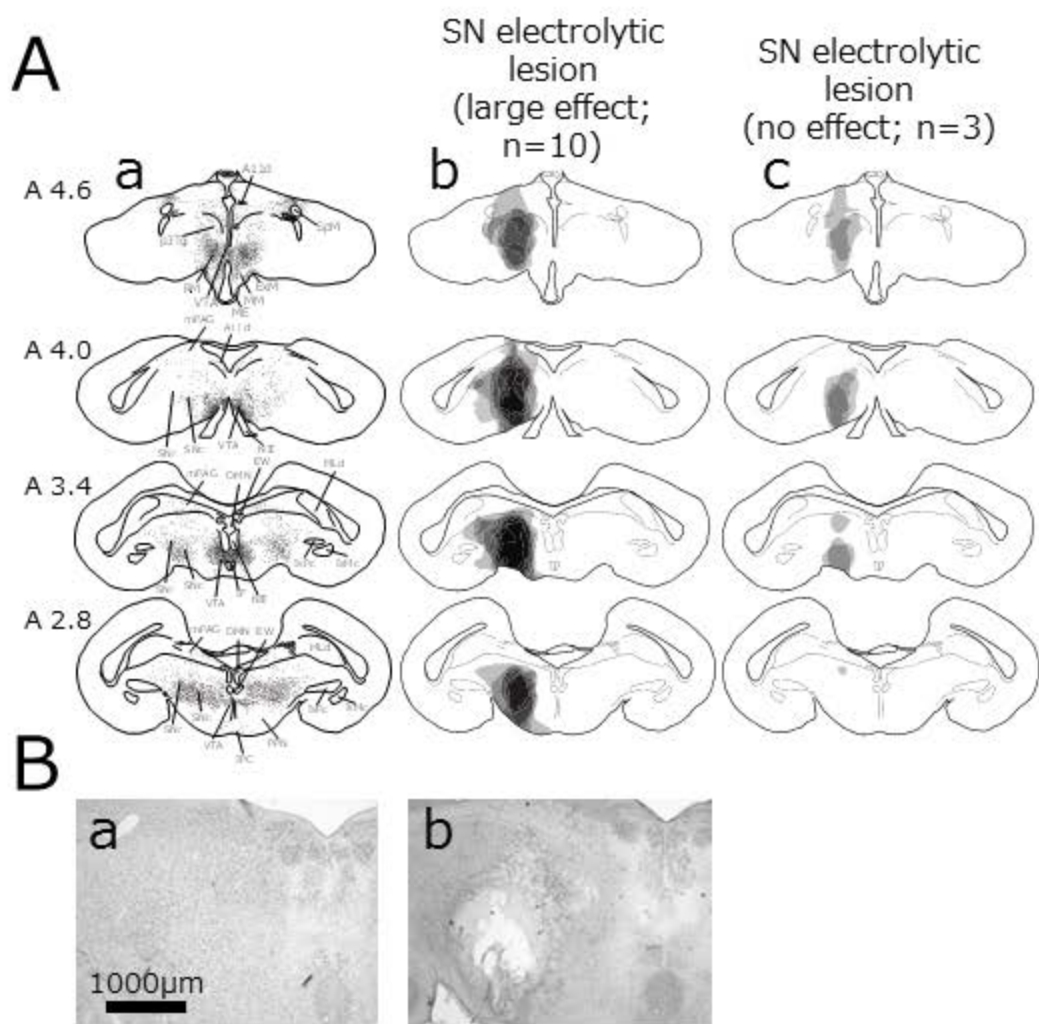


Figure 7

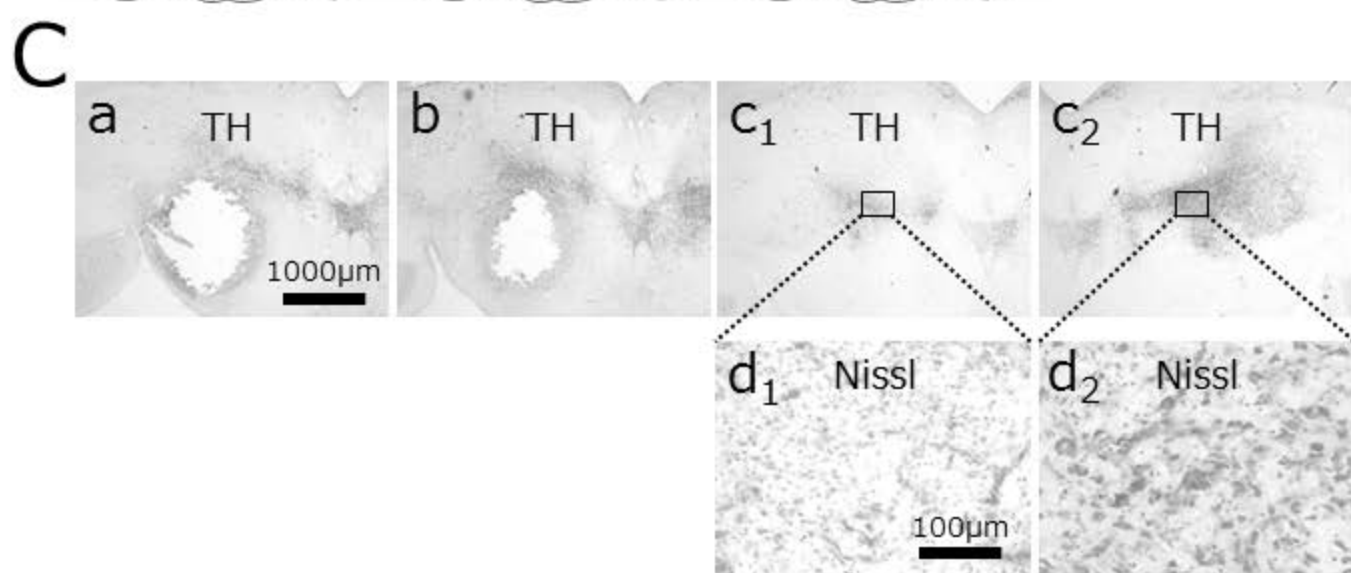
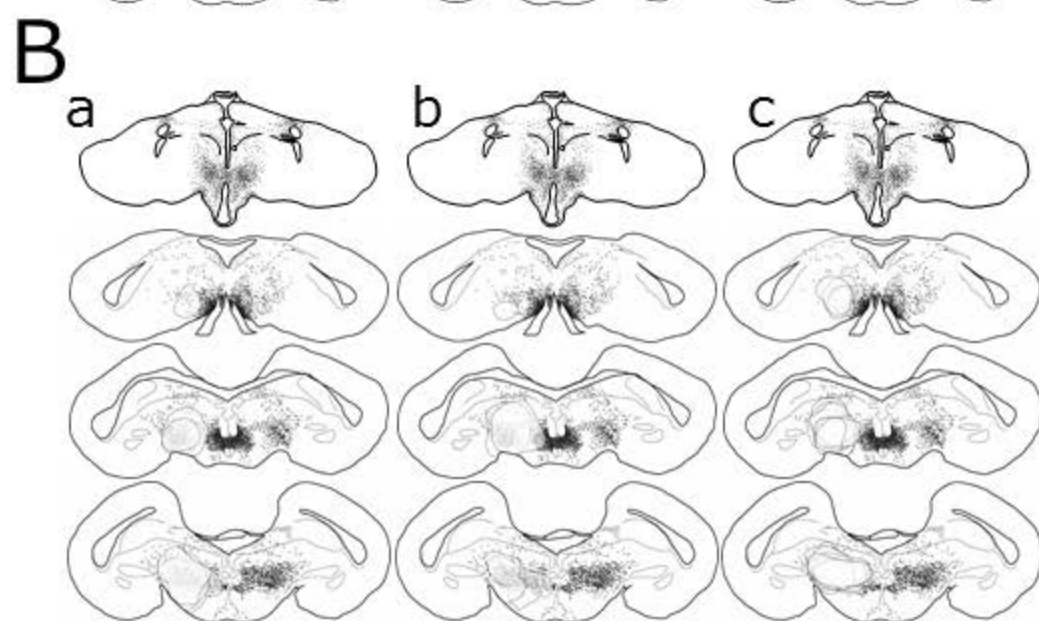
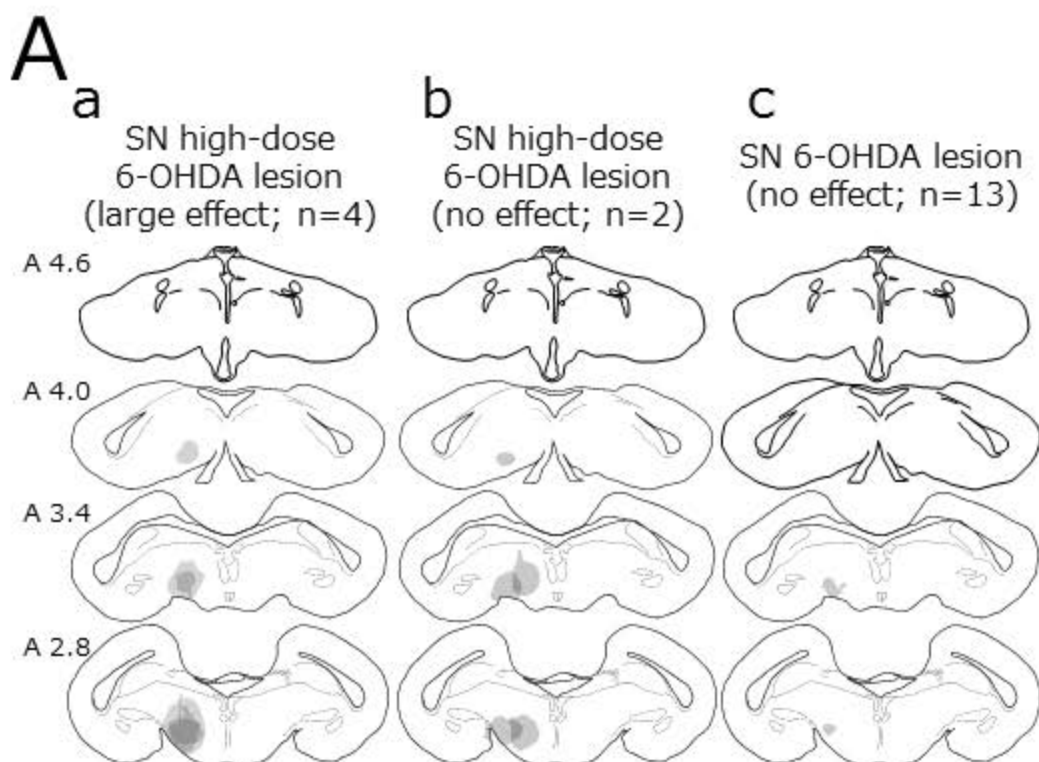
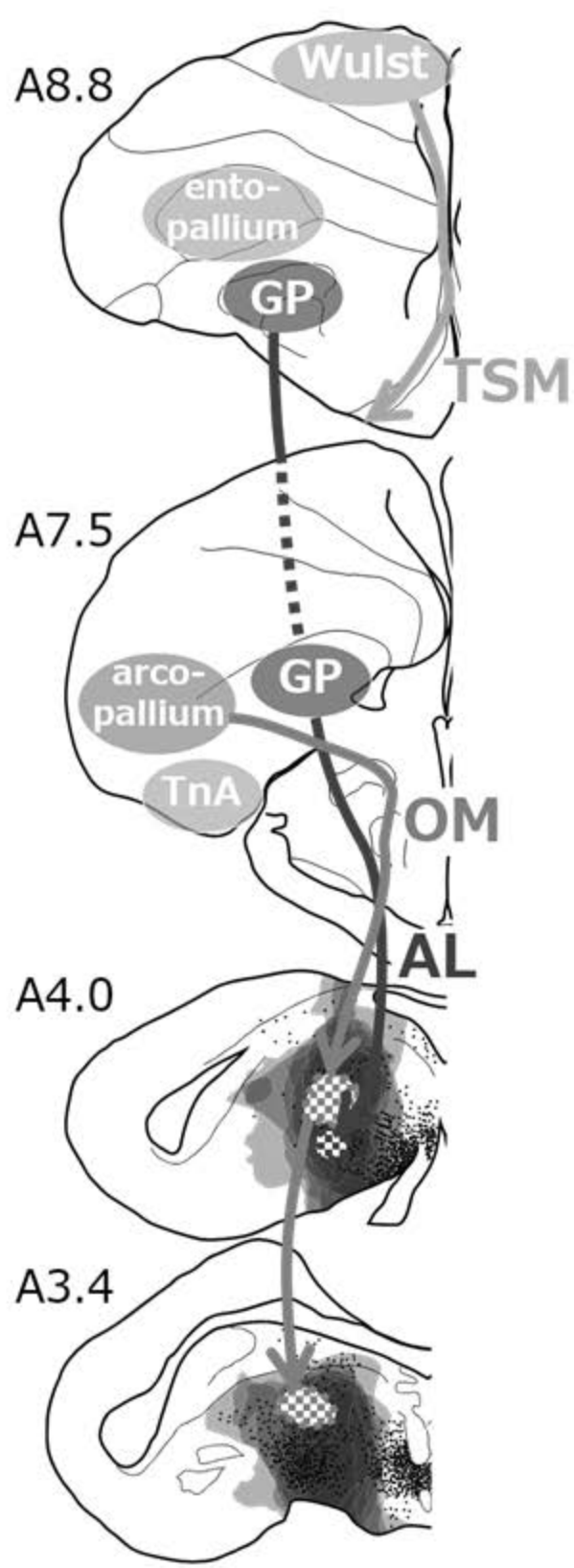


Figure 8



Mesolimbic and nigrostriatal pathways of the domestic chicks:
dissociation of two major ascending dopaminergic projections
revealed by factor analysis on the TH immunostaining
after localized infusion of 6-OHDA to midbrain tegmentum

As has been described in Introduction, major dopaminergic projections ascending from midbrain nuclei have been reported to comprise two pathways, each with distinct terminal areas in the telencephalon. One is the mesolimbic pathway from VTA, and another is the nigrostriatal pathway from SN (Durstewitz et al. 1998, 1999, Csillag 1999, Reiner et al. 2004).

We initially tried to ablate each pathway by placing localized lesions in midbrain nuclei. However, selective lesioning proved to be difficult, as TH-positive neurons form a continuum spanning from VTA to SN (see Fig. 5A of the main text). Actually, infusion of 6-OHDA to SN reliably ablated most of TH-positive neurons in SNc and SNr, while sparing VTA neurons intact. On the other hand, in all chicks in which 6-OHDA infusion was targeted at VTA, we found that (1) considerable part of the SN (particularly SNc) showed a suppressed TH immuno-positivity, and (2) some TH-positive neurons remained undamaged in VTA. Examples are shown in Figure S1A and B.

In the chick shown in A, unilateral infusion was targeted at SNc-SNr of the right hemisphere, and the right VTA was left intact (* in upper traces of frontal sections in A). Accordingly, the TH-positivity in LSt, GP and MSt (Aa) was suppressed, but staining in NAc, septum, IMM and BSTl were comparable to the respective contralateral counterparts. It is to be noticed that Arco and IMM on the infusion side also remained intact.

In the chick B, unilateral infusion was targeted at VTA of the right hemisphere, but SNc – SNr also showed a suppressed TH-positivity (** in upper traces in B). In this chick, TH-positivity in MSt was completely lost (Ba), and stronger suppression occurred in NAc, BSTl and septum. Notice that a number of TH-positive cell bodies were found even in the close vicinity of the infusion site in VTA (photos Bc). Non-selective tissue damages did not occur at a moderate dose, but the depletion of catecholaminergic neurons was incomplete.

To access the degree of overlap and segregation, we examined how TH-positivity was correlated among telencephalic areas of the infusion side. In each area, degree of suppressed

TH-positivity varied among chicks, because population of the catecholaminergic neurons damaged by the 6-OHDA infusion varied. If the staining in area X was highly correlated with that in area Y, we would assume that X and Y receive projections from common set of catecholaminergic neurons. If, on the other hand, the correlation between X and Y was poor, we would assume that these areas receive separate projections.

In 29 chicks that received a unilateral 6-OHDA infusion to midbrain, TH immunostaining was systematically compared between bilateral hemispheres. Of these 29 chicks, behavioral changes were examined in 19 chicks (6 with a high-dose, and 13 chicks with a moderate dose 6-OHDA). No behavioural tests were accomplished in the remaining 10 chicks, in which 6-OHDA infusion was targeted at VTA (2 with a high dose and 8 with a moderate dose). These chicks survived for *ca.* 5 days after the 6-OHDA infusion, and their brains were processed for TH immunohistochemistry.

Brain area was scored as “2” if the TH-positivity was completely diminished (Figure S1; MSt in the photo Ba). A region was scored as “0” if no detectable TH depletion occurred (NAc and BSTl in the photo Aa), and partial depletion was scored as “1”. We subsequently constructed a matrix in which 29 individual chicks (columns) x 14 telencephalic brain areas (lines) were plotted, and each cell of the matrix contained the score (0, 1 or 2). From this matrix (not shown), we calculated the degree of correlation in all pairs out from the 14 brain areas (105 pairs in total). In Figure S2A, those pairs with high degree of correlation are coded as dark rectangles, and those with low degree are as lighter rectangles.

Based on the correlation data, by using the method of factorial analysis (DeCoster 1998; Revelle 2013), we developed a simple model in which minimal number of factors parsimoniously determine the observed pattern of correlations. As shown in Figure S2B, two factors (a_1 and a_2) are accessed to be appropriate to explain the TH-positivity patterns of 14 brain areas in these 29 chicks. It is natural to assume that a_1 represents the dopaminergic neurons in SNc-SNr, while a_2 represents those in VTA.

We noticed that considerable overlaps occurred in MSt, suggesting that MSt receive projections from both pathways. In addition, correlations between Arco vs. lateral areas (such as mLSt / GP / LFB) were low. Correlation was similarly low between septum vs. IMM. It is therefore possible that Arco, septum and IMM could receive projections from catecholaminergic sources other than these two dopaminergic nuclei.

In conclusion, it is reasonable to assume a topology of the midbrain catecholaminergic projections to telencephalon organized along the medio-lateral axis, with some overlaps in MSt and other nuclei. The MSt lesions should thus impaired ascending projections from the two pathways.

Supplementary figure legends

Figure S1. TH immuno-positive cell bodies and fiber terminals in two example chicks. **A:** infusion of 6-OHDA (moderate dose) in SNc; track of the inserted syringe in the photo Ac. **B:** infusion of 6-OHDA (moderate dose) in VTA.

Figure S2. Factorial analysis of the projection patterns based on the degree of correlations between pairs of telencephalic areas. **A:** The degree of correlation in TH immuno-positivity was plotted in the 105 pairs. Darker rectangles denote the pairs with high correlation. **B:** Two factors (a_1 and a_2) explain the observed patterns of TH-immuno-reactivity. Thick lines suggest dense projections, whereas thin lines weaker ones.

Abbreviations

6-OHDA	6-hydroxy dopamine
Arco	arcopallium
BSTl	lateral part of the bed nucleus of stria terminalis
ExA	extended amygdala (see Bálint et al. 2011)
GP	globus pallidus
IMM	intermediate medial mesopallium
LSt	lateral striatum
LFB	lateral forebrain bundle (see Bálint et al. 2011)
ILSt	lateral part of LSt
LSt	lateral striatum
MSt	medial striatum
NAc	nucleus accumbens
post BSTl	posterior part of BSTl
SN	substantia nigra

SNC	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
TH	tyrosine hydroxylase
VTA	ventral tegmental area
VP	ventral pallidum

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