Neuroethological studies of social facilitation in domestic chicks

(ニワトリ雛の社会的促進に関する
神経行動学的研究)

A DISSERTATION
submitted to the Graduate School of Life Science,
Hokkaido University
in partial fulfillment of the requirements for the degree of
DOCTOR OF LIFE SCIENCE

Yukiko Ogura

2014

学 位 論 文 内 容 の 要 旨

博士（生命科学） 氏 名 小倉 有紀子

学 位 論 文 題 名

Neuroethological studies of social facilitation in domestic chicks
(ニワトリ雛の社会的促進に関する神経行動学的研究)

【序論】
本学位論文は三章から構成される。第一章では新たな行動課題を開発して行動薬理学的実験を行い、採餌のための運動（エフォート）に対する神経修飾物質セロトニンおよびドーパミンの役割を調べた。第二章では定量的な行動解析に基づき、社会採餌下でエフォートが増えること、特に、この社会的促進は採餌量の増大を伴わなくとも生じることを明らかにした。第三章では局所的な脳破壊実験を行い、内側線条体と黒質が採餌エフォートとその社会的促進に関して二重に分離した機能を備えていることを明らかにした。ドーパミンの社会的促進に果たす役割についても検討した。

【第一章】採餌エフォートに対するセロトニンとドーパミンの役割
神経修飾物質であるセロトニンは、餌報酬に対する時間コストの決定に寄与すると広く考えられている（Doya 2008 for review）。他方、運動（エフォート）コストの決定への効果は十分に調べられていなかった。
本章では神経薬理学的に脳内セロトニン濃度を上昇させた条件下で、ニワトリ雛の採餌エフォートを調べた。採餌エフォートを定量化するため、両端に餌場のあるI字迷路を用いた。変動間隔スケジュールで給餌を行う間、雛は餌場間を往復し続けた。この往復運動量と走行速度を指標として、選択的セロトニン再取り込み阻害薬フルボキサミンの効果を調べた。フルボキサミンはセロトニン輸送体と結合してその働きを阻害し、シナプス間隙のセロトニン濃度を高めることが知られている。フルボキサミン投与群とビークル群とで往復運動量を群間比較した。フルボキサミン投与群はビークル群に比べ、往復運動量および走行速度が低かった。続いて、セロトニンの主要な投射先である内側線条体をターゲットに、脳内微小透析法により神経修飾物質の放出量の変化を調べた。フルボキサミン投与によりセロトニン濃度が増大すると共に、ドーパミン濃度も微増することが判明した。
以上より、内側線条体におけるセロトニン・ドーパミン相互作用が採餌エフォート量の決定に寄与することが示唆された。

【第二章】採餌エフォートの社会的促進に関する行動学的研究
社会的促進は他者による行動量の増大全般を指し（Zajonc 1965 for review）、ヒトからアリまで幅広い動物種で報告されている。採餌行動に限っても、その社会的促進は多くの脊椎動物で知られているが、先行研究では餌を無制限に与えていた。そのため餌量も増大し、それが副次的にエフォート量を増大させていた可能性が否定できなかった。
本章ではまずI字迷路において単独採餌ないしはペア採餌をさせ、往復運動量を群間比較した。ペア採餌群の運動量は単独採餌群に比べて高くなった。飼育ケージにおける社会採餌の経験は無関係であった。次いで餌をめぐる競合の影響を除くため、雛および餌箱を透明ないしは不透明な仕切り板で分断した。仕切りが透明な（ペア相手が見える）条件であれば、餌の競合がなくても往復運動量は増えた。他方、仕切りが不透明な（ペア相手が見えない）条件では、餌の競合の有無に関わらず、往復運動量は増えなかった。同様の社会的促進は採餌の完了行動（餌を採るためについばむ運動）にも生じ、この場合も獲得さ

れる餌量は増えていなかった。以上より、採餌行動の社会的促進は、餌量の増大によらず、他者を視覚的に認める事だけで生じることが判った。

【第三章】採餌エフォートとその社会的促進に対する内側線条体・黒質の機能的分離

社会的促進の神経機構はこれまで、まったくと言って良いほど調べられていなかった。そこでまず、社会的促進が採餌エフォートに対して生じることから、採餌エフォートの決定に係る神経系が関与すると仮定して、一連の局所脳破壊実験を行った。第一章から得られた知見を基に、中脳腹側被蓋野から内側線条体へのドーパミン性投射（内側系）に着目した。他方、第二章で論じたように、社会的促進は餌量の増大が伴わなくとも生じる。よって社会的促進の系は、餌報酬に基づいて採餌エフォートを決定する系とは独立している可能性もある。そこで、もう一つの有力なドーパミン性神経投射である、中脳黒質から外側線条体への系（外側系）にも着目した。内側系とは解剖学的に分離しており（Mezey & Csillag 2002）、哺乳類においては機能的にも分離していることが指摘される（Balleine & O'Doherty 2010）。しかし、社会的促進との関連については不明であった。

内側系については内側線条体、外側系については黒質を標的部位とし、電気破壊ないしはドーパミン性神経選択的破壊の効果を偽処置群との群間比較により検討した。電気破壊の効果はニッスル染色標本により検討した。さらにドーパミン作動性神経細胞と終末への破壊の効果は、TH（チロシン水酸化酵素）免疫組織化学染色法により検討した。1 回のテストのなかで、被験個体には単独採餌とペア採餌の双方を連続的に経験させ、両者を比較することによって社会的促進を調べた。採餌エフォート量の指標として単独時の往復運動量、社会的促進の指標としてペア時と単独時との往復運動量の差分を用いた。対照群（偽処置群）の結果から、これらの指標は互いに独立であると考えられた。

単独時の往復運動量（採餌エフォート量の指標）は、内側線条体電気破壊群のみ、偽処置群に比べて低下した。ドーパミン性神経破壊の効果は見られなかった。黒質については電気破壊・ドーパミン性神経破壊ともに無効であった。他方、往復運動量の差分（社会的促進の指標）は、黒質電気破壊・ドーパミン性神経破壊によって小さくなった。内側線条体破壊の効果は見られなかった。

更に、行動指標を基に破壊領域を 1 個体ごとに検討し、影響が大きい脳領域を検討した。内側線条体では背側部が破壊された個体において、往復運動量に対する影響がより大きい傾向がみられた。黒質では外背側部が破壊された個体において、社会的促進に対する影響が大きい傾向が見られた。ただし、内側線条体においては組織の機械的破壊を伴わず、ドーパミン性神経終末のみの破壊に留まったのに対し、黒質においては機械的破壊が生じていた。黒質のドーパミン性神経破壊については、更にデータを追加する必要がある。

以上から、腹側被蓋野-内側線条体系と黒質-外側線条体系が異なる機能を持つことが示唆された。すなわち、前者は採餌エフォートの決定に、後者はその社会的促進に寄与し、両者は二重に分離していると考えられる。但し内側系の機能的分離を確実に示すためには、腹側被蓋野の破壊によっても社会的促進が減弱しないことを示す必要がある。

【考察】

社会的促進という現象は Triplett （1898）がヒトについて記述して以来、一世紀以上にもわたって心理学の重要なトピックであった。にもかかわらず、神経生物学の観点から次の二点が未解決のままであった。第一に報酬（採餌量）と因果的に結びついているかという問題、第二が何を神経基盤とするかという問題である。本研究では社会的促進の定量的解析に適した行動課題を新たに開発し（第一章）、社会的促進が採餌量の増大を伴わなくとも起こる事を示し（第二章）、さらにその神経基盤が採餌エフォートの制御系と独立であることを示した（第三章）。社会的促進に寄与する脳領域を同定したのは本研究が初めてである。しかしドーパミンの関与については、今後更なる検討が必要である。

CONTENTS

CHAPTER I

Effect of serotonin for foraging effort

in domestic chick

1 Introduction

Because food items are unevenly distributed in nature, animals often forage by sequentially visiting one patch after another. As the food density of a patch gradually declines, the energy intake rate should inevitably decrease. Foragers should thus leave the depleting patch at an appropriate point, even though some food items are remaining. By adopting marginal value theorem, Charnov (1976a) argued that optimal foragers leave a patch at the point when the instantaneous gain rate matches the average gain rate available in the environment. The optimal patch-use model, as formulated by Stephens & Krebs (1986), gained strong empirical support through studies of diverse animals in the wild. For example, wasps (Kasuya 1982) and starlings (Kacelnik 1984) have been shown to behave optimally so that the long-term gain rate was maximized in the central-place resource collection.

Some studies have tried to examine patch-use behaviors as psychological tasks performed in the laboratory. Agetsuma (1998) simulated a depleting food patch by gradually decreasing reinforcements, and found that the subject monkeys stopped responding in a manner that matched the optimal patch-use model. On the other hand, Hayden et al. (2011) simulated a patch-use condition and analyzed neural activities of the dorsal anterior cingulate cortex. In Hayden's protocol, monkeys made choices between an option after a fixed short delay (gradually smaller drops of juice, mimicking next food in the patch) and an alternative option after a variable "travel time" (reset of the diminished return, mimicking new patch). In that study, however, the prospective "travel time" was explicitly presented, and the monkeys could make decisions in a manner similar to the inter-temporal choice paradigm. In the concurrent choices,

animals generally adopt a short-sighted strategy such as maximization of instantaneous gain rate (Mazur 1981) or profitability (Matsushima et al. 2008), in contrast with the paradigm of patch-use behavior.

The discrepancy between these two paradigms was clearly pointed out by Stephens and Anderson (2001), who examined captive blue jays under two different conditions, one mimicking patch-use behavior ("stay or leave" choices on single options), and the other inter-temporal choice (concurrent choices of "small-immediate" and "large-delayed" options). Even though the economic consequences of these two conditions were set as identical, the jays behaved differently and showed a considerable far-sightedness in the patch-use task. The authors thus argued that behavioral data obtained in the inter-temporal choice paradigm are not an "accurate guide" for understanding patch-use behavior. However, it remains unanswered whether the neural substrates are distinct or shared between these two paradigms of behavior.

In the present study, we examined if these paradigms could share serotonin (5-HT) as a common neuro-modulator. In inter-temporal choices tested in rats, antidepressant drugs (imipramine-like drugs and serotonin reuptake inhibitors) have been shown to acutely improve impulse control, suggesting the involvement of noradrenergic / serotonergic neuro-modulation (Bizot et al. 1988, 1999); null results (Charrier & Thiébot 1996, Evenden & Ryan 1996) should also be noticed. However, 5-HT depletion by 5,7-dihydroxytryptamine infusion to raphe nuclei caused rats to commit more impulsive choices than controls (Wogar et al. 1993, Mobini et al, 2000), supporting the critical role of 5-HT. Functional involvement of 5-HT has also been shown by Denk et al. (2005), where a blockade of 5-HT synthesis caused rats to choose an immediate reward more frequently without effects on effort-based choices. Taking

these experimental findings into account, Doya (2008) proposes an integrated framework in which neuromodulators (5-HT, dopamine and noradrenalin) affect economical decision making. However, the involvement of modulators has not been studied in patch-use behavior, except for a recent genetic study in *Caenorhabditis elegans* (Bendesky et al. 2011).

Fluvoxamine maleate (FLV) was used as the selective serotonin reuptake inhibitor (SSRI) in this study, because FLV binds to a chicken's 5-HT transporter (SERT) at a high affinity, comparable with the human SERT (Larsen et al. 2004). If 5-HT level is increased by FLV application and if choice impulsiveness is suppressed by FLV, then FLV may also influence patch-use behavior. FLV-treated chicks may leave a depleting food patch earlier, so that they gain a larger amount of food after a longer delay (or a new patch after a long travel time) as illustrated in Fig 1. In addition, the effects of FLV on work efforts and distress calls were also measured.

Pharmacological effects of FLV on the striatal 5-HT level were examined by *in vivo* microdialysis. Striatal nuclei (neostriatum and nucleus accumbens [NAc] in particular) have been shown to play a critical role in inter-temporal choices, because localized lesions caused impulsive choices in rats (Cardinal et al. 2001) and in domestic chicks (Izawa et al. 2003). Dense 5-HT innervation of the caudate putamen by the dorsal raphe is reported in rats (Waselus et al. 2006). In chicks, similarly, NAc and the surrounding striatum are similarly innervated by 5-HT positive fibers (Metzger et al. 2002).

1.2 Materials and Methods

1.2.1 Animals

A total of 193 male domestic chicks (*Gallus domesticus*, white leghorns) were used. Each chick was used once and never reused in other experiments. Thirteen chicks were discarded because they heavily emitted distress calls, therefore the present study was based on data obtained from the remaining 180 individuals; 36 chicks (Experiment 1), 70 (Experiment 2), 20 (Experiment 3), 16 (Experiment 4), 18 (Experiment 5), 12 (Experiment 6), and 8 (Experiment 7), respectively. Five additional chicks served as companion individuals in the study of distress calling. New hatchlings (post-hatch day 1) were purchased from a local supplier (Hokuren Central Hatchery, Iwamizawa, Hokkaido) and were communally housed in transparent plastic cages (15 × 28 × 12 cm) that were thermo-controlled at *ca.* 30°C under illumination by white light bulbs (12L: 12D, the light period starting at 08:00). On days 2–4, chicks were fed with 1–3 g of food (per day per chick; mixture of millet and chick mash food). On day 5 and afterwards, chicks were supplied with a daily 4 g ration in addition to 0.5–1.0 g of millet gained during experiments. Water was freely available. Body weight steadily increased, maintaining *ca*. 80% of the freely-fed body weight. After the end of the experiments, chicks were sacrificed by carbon dioxide if not processed for brain histology. Experiments were conducted under the guidelines and approval of the Committee of Animal Experiments of Hokkaido University. The guidelines are based on the national regulations for animal welfare in Japan (Law for the Humane Treatment and Management of Animals; after a partial amendment No.68, 2005).

1.2.2 Apparatuses and drugs

1.2.2.1 I-shaped maze for measurement of patch residence time

An I-shaped maze was used for recording the residence time (Experiment 1 and 2, Figure I-2A). The maze was composed of a pair of feeders connected by a runway (60 cm long and 13 cm wide). Each feeder was equipped with a mechanical sensor and a colored door that was painted in blue or red. One of the feeders served as *ON* feeder that supplied grains of millet. Color assignment was counter-balanced among individuals in each group. The sensor detected the arrival of a chick and triggered the door to open for the chick to approach the food tray. The *ON* feeder delivered food (1 or 2 grains of millet at a time) at pre-programmed intervals shown up to 25 times. Figure I-2B (a and b) illustrate the patterns of gradually diminishing food supply by plotting the cumulative gain (number of millet pellets) against the residence time (sec) during which the chick stayed at the *ON* feeder. Open and filled circles denote the time at which food of 1 (a) or 2 (b) grains was delivered. For further explanation of the procedures of behavioral tasks, see below (section 2.3.1).

The opposite (*OFF*) feeder did not deliver food. Visits to the *OFF* feeder terminated the food supply at the *ON* feeder, and caused the interval between grain deliveries at the *ON* feeder to be reset to the minimum value. Programmed food supply at the *ON* feeder was restarted when the chick revisited the *ON* feeder (Figure I-2C). Chicks quickly learned to forage food by shuttling between the two feeders. The I-maze was placed in a dark room, illuminated by white light bulbs, and kept at *ca.* 26–30°C. Behavior was monitored by a video camera placed above the maze, so that experimenters observed chicks without being seen.

1.2.2.2 Operant chamber for binary choice tests

An operant chamber was used for recording behaviors in the inter-temporal choice paradigm (Experiment 3). A thermo-controlled box (21 × 19 × 25 cm, kept at *ca.* 27–30°C and illuminated by light bulbs) was used (Aoki et al. 2006b, Amita et al. 2010, Amita & Matsushima 2011). One of the surrounding walls was equipped with a pair of holes placed side by side (separated by 3 cm, 4 cm above floor level), through which one or two colored beads (white, blue or red) were presented for 1 sec. When a chick pecked one bead, both beads were withdrawn and millet food (1 or 6 grains) was supplied to the central food tray after a delay (0 or 1.5 sec). Assignment of colors to small/short-delay food (red for 1 grain after 0 sec; ***SS***) and large/long-delay food (blue for 6 grains after 1.5 sec; ***LL***) was fixed among individuals. A white bead was always assigned to a non-rewarding option.

1.2.2.3 I-shaped maze for studying work efforts

An I-shaped maze (88 cm long and 12 cm wide) was used for testing each chick's foraging efforts (Experiment 4); see Ogura & Matsushima (2011) for details. Briefly, the maze was equipped with a pair of terminal feeders, each of which supplied a grain of millet at variable intervals (mean = 15 sec, uniformly ranging 10–20 sec) for 30 times (therefore 60 grains in total). Note the low reinforcement rate, which did not deplete during the trial that lasted for 8–9 min. The maze was not equipped with doors, and chicks freely shuttled between the two feeders and gained food if available. No visual cues were delivered but distracting motor sounds were given so that chicks were unable to associate any sensory cues with food delivery. Behavior was recorded by a video camera placed above the maze, and trajectories of the shuttling chick were analyzed off-

line by using Move-tr/2D (ver 7.0) software (Library Co., Japan) according to the position of a fluorescent marker attached to the head.

Other behavioral tests (distress calls; Experiment 5) and *in vivo* microdialysis (Experiment 6 and -7) were conducted in the operant chamber, but chicks did not perform any specific behavioral tasks.

1.2.2.4 Drugs

Fluvoxamine maleate (FLV) was kindly supplied by Abbott Japan Co., Ltd. (former Solvay Pharmaceuticals B.V.) / Meiji Seika Pharma Co., Ltd. FLV was dissolved in a 0.01 M phosphate-buffered saline (pH 7.2) at concentration of 1.5 mg or 3.0 mg per ml, and intra-peritoneally administered at 1 ml/15 g body weight (BW), yielding 10 or 20 mg/kg BW, respectively. For *in vivo* microdialysis experiments (Experiment 6 and -7), we used an artificial cerebrospinal fluid (aCSF) composed of: KCl 2.7 mM, NaCl 140 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.0 mM, NaH_2PO_4 0.03 mM, Na_2HPO_4 1.7 mM, pH 7.2. In the reverse microdialysis (Experiment 7), FLV was added to the aCSF at concentrations of 2 or 20 μM.

1.2.3 Procedures of behavioral tasks

1.2.3.1 Patch residence time (Experiment 1 and -2)

On post-hatch day 8, chicks were initially given 3 consecutive blocks of habituation to the I-maze. In each block, a communal group of 3 individuals was placed in the maze with 25 grains of scattered millet for 15 min. On days 9–11, individual chicks were tested in a block of behavioral sessions per day, and a block was composed of 20 cycles of shuttling between the *ON* and *OFF* feeders. Chicks were discarded if

they stopped midway continually emitting distress calls for a period longer than 15 min.

We recorded behaviors under 6 different patterns of food supply (Figure I- 2B). To avoid effects of preceding experiments, each chick was examined under one condition and not reused. In 4 patterns, the *ON* feeder delivered 1 grain of millet at a time, and up to 25 grains were supplied. In 2 other conditions, 2 grains were delivered at a time, so that the gain was 50 grains in total.

Intervals between delivery was either kept constant ({2.0, ×1.0} and {14.0, ×1.0} conditions) or gradually increased ({2.0, ×1.1} and {0.5, ×1.2} conditions). Figure I-s in parentheses {α, xβ} indicate that the initial interval (between the 1st and 2nd delivery) was α sec, and increment rate was β. The last interval (between the 24th and the 25th) of the depleting conditions was therefore 16 and 22 sec for {2.0, ×1.1} and {0.5, ×1.2}, respectively. Predictions of these experiments are schematically illustrated in Figure I-3A and B.

Four parameters (periods of time) were measured based on the sensor signals recorded by a PC using the LabView system (National Instruments Co., USA). (1) ***Residence time*** in the *ON* feeder: period from the arrival at the *ON* feeder until the arrival at the *OFF* feeder. (2) ***Leave-point interval***: interval between the last two deliveries at the point when the chick left the *ON* feeder (Figure I-4C). (3) ***Travel time***: period from the arrival at the *OFF* feeder until the arrival at the *ON* feeder. (4) ***Waiting time***: period from the leave point until the point of time at which the next grain should have been delivered in the *ON* feeder (Figure I-4D).

As the first step, behaviors were recorded without any pharmacological treatments in 6 groups of chicks (Experiment 1; under 4 diminishing conditions and 2 constant interval conditions, as described above). Behavioral tasks were conducted for 3

days from post-hatch day 9 to day 11. Chicks were examined in one block (20 shuttles) per day.

As the second step, the effects of FLV on *residence time* were examined in 6 other groups of chicks (Experiment 2); vehicle control, 10 and 20 mg/kg BW in each of the 2 diminishing conditions. Behavioral tasks were conducted for 4 days from post-hatch day 9 to day 12. On day 9, one block (composed of 30 shuttles) was given, and chicks were pseudo-randomly allocated into 3 groups (vehicle, 10 and 20 mg/kg BW); 3 groups were thus counter-balanced based on the recorded residence time. On days 10–12, chicks received one block (composed of 20 shuttles) per day, 1 hour after the FLV or vehicle solution was intra-peritoneally injected.

1.2.3.2 Inter-temporal choices

Effects of FLV on inter-temporal choices (Experiment 3) were examined according to the procedure described previously (Amita et al. 2010). Briefly, after initial habituation to the operant chamber on day 5, chicks were individually trained to associate color cues with food rewards as described above. One block of single-cue training consisted of 12 pseudo-randomly arranged trials for ***SS*** (small/short-delay reward; red), 12 trials for ***LL*** (large/long-delay reward; blue), and 24 trials for no reward (white), respectively. Chicks received 2 blocks of training on day 6. On days 7–9, chicks were thereafter trained in binary choices between a rewarding bead (red or blue) and a non-rewarding white bead in one block per day. One block consisted of 12 pseudo-randomly arranged trials for ***SS*** (red) paired with white, 12 trials for ***LL*** (blue) paired with white, and 24 trials for a pair of non-rewarding white beads. The side of presentations was randomised in each trial. On day 10, the remaining chicks were

allocated to two groups in a counter-balanced manner based on body weight; one group for FLV and another for vehicle treatment. At 60 min after the injection, chicks were tested in one block composed of 10 test trials of choices between ***SS*** (red) and ***LL*** (blue), which were pseudo-randomly arranged with trials for a pair of non-rewarding white beads (10 trials), a pair of ***SS*** (red; 10 trials) and a pair of ***LL*** (blue; 10 trials).

1.2.3.3 Work efforts in uncued shuttle tests

Effects of FLV on work efforts (running for feeders) were examined (Experiment 4) according to the procedure described elsewhere (Ogura & Matsushima 2011). Briefly, chicks were habituated to the I-maze on days 7 and 8, and then allocated to two groups in a counter-balanced manner based on body weight; one group for FLV and another for vehicle treatment. On days 9–11, chicks were individually tested 75 min after the FLV or vehicle solution was intra-peritoneally injected. In each test session, the two terminal feeders supplied millet grains according to the programmed food delivery that lasted for 8–9 min in total. After the food supply was terminated and all grains were consumed, chicks were left in the maze for an additional 2 min before the test session of the day was terminated.

Five parameters were measured. (1) ***Total Residence time*** (sec) in each feeder (*i.e.*, areas < 10 cm from the terminal walls). (2) ***Number of visits*** (times) to each feeder. (3) ***Residence time per visit*** (sec/times) (*i.e.*, the ***residence time*** divided by the ***number of visits***). (4) ***Running distance*** (cm) and (5) ***velocity*** (cm/s) during the 8 min period of food delivery; ***Velocity*** was measured in the midway after excluding the areas near (< 10 cm) the feeders.

1.2.3.4 Distress calls

Effects of FLV on distress calls were examined at 3 doses (vehicle, 10, and 20 mg/kg BW, Experiment 5). To induce distress calls, the subject chick was socially isolated from companion chicks for 3 min, as reported previously (Hayashi et al. 2001), and the number of distress calls were counted. Calls were also counted before and after the isolation period (the average yielded baseline level, each for 3 min), and the difference (***Δ distress calls***) was calculated. Calls were counted 20 min after the FLV or vehicle injection. FLV or the vehicle was injected every day from day 8 to day 10, and calls were measured on days 8 and 10.

1.2.3.5 Statistical analysis

For the data obtained in Experiments 1 to 4, generalized linear mixed models (GLMMs) were constructed to estimate the effects of behavioral factors and the drug application by using R (ver. 2.6.0) for the platform of statistical calculations. See Table I-1-3 and supplementary tables for details. For the distress calls (Experiment 5), ANOVA was used after Bartlett's test for homogeneity of variance with the significance level set at $p < 0.05$.

1.2.4 Microdialysis

1.2.4.1 Surgical operation for chronic implantation of cannulae

Effects of systemic injection of FLV on centrally released serotonin (5-HT) and dopamine (DA) were examined in Experiment 6 by microdialysis. To confirm its direct effects on the central nervous system, the effects of locally infused FLV through the dialysis microprobe (reverse microdialysis) was examined in Experiment 7.

A guide cannula was chronically implanted on the day before the microdialysis experiment. Chicks were anaesthetized by intra-muscular injection of ketamine and xylazine (0.24 ml of a 1:1 mixture of 10 mg/ml ketamine (Sankyo Co., Japan) and 2 mg/ml xylazine hydrochloride (Sigma, USA). Supplementary injections (0.05 ml each) were given to maintain a stable anaesthesia if necessary. Chicks were then fixed on a stereotaxic apparatus. Skin over the skull was incised, and the skull bone and dura matter were cut out to expose the brain surface. A guide cannula (AG-8, Eicom Co., Japan) was inserted into the medial striatum (mSt) of the left hemisphere. Coordinates of the probe tip were; 1.20–1.75 mm anterior from the bregma, 1.2–1.5 mm lateral from midline, and 4.3–5.3 mm from brain surface. Incised skull was covered with cotton, and the cannula was secured to the skull with dental cement. In Experiment 6, a polyethylene tubing (1.0 mm o.d.) was further inserted into the peritoneal body cavity for systemic injection of FLV. The tubing was implanted under the skin and connected to a 1 ml syringe.

1.2.4.2 Sampling perfusate and high performance liquid chromatography (HPLC) measurement of 5-HT and DA

On the next day, an *in vivo* microdialysis experiment was conducted in a freely-behaving condition without any behavioral tasks. A dialysis probe (A-I-8-02, Eicom Co.) was inserted into the brain through the guide cannula, and sampling started immediately. The active membrane of the probe (0.22 mm o.d., 2 mm long) was exposed to brain tissue. The internal space of the probe was perfused with aCSF at a rate of 1 μl/min using a syringe pump (ESP-32, Eicom Co.). Contents of 5-HT and DA in the perfusate were measured by HPLC with a reverse-phase column (PP-ODS, Eicom

Co.) and an electrochemical detector (ECD-300, Eicom Co.). The mobile phase consisted of: 2.1 mM sodium 1-decansulfonate, 0.1 mM EDTA-2 Na, 0.1 M phosphate buffer and 1% (v/v) methanol (pH 6.0). A working electrode was maintained at +400 mV, and the flow pump rate was set at 0.5 ml/min. A typical example of HPLC chromatogram is shown in supplementary Figure I-S1; dopamine and 5-HT were clearly separated without overlapping. Standard curve (supplementary Figure I-S2) indicated that the measured signal (AUC; area under cover, in min x nA) was proportionate to the concentration of dopamine and 5-HT in a range of 0.01 to 1 ng/ml, which covered the range of perfusate samples obtained in this study. For statistical comparisons, non-parametric tests were used at a significance level set at $p < 0.05$.

1.2.4.3 Histological verification

After the microdialysis experiment, the probe location was marked by injecting dye (0.1% pontamine sky blue, ca.10 µl). The dialysis probe was replaced with another probe with a small hole at the tip, and the dye was injected by pressure. Chicks were anaesthetized by an injection of ketamine – xylazine cocktail (0.5 ml of a 1:1 mixture), and transcardially perfused with a fixative (4% paraformaldehyde in 0.1 mM PB, pH 7.2, 50 ml). Brains were dissected out of the surrounding tissue, post-fixed at 4°C for 1–7 days, embedded in egg yolk, and cut into 50 µm thick frontal sections by using a vibrating microslicer (DTK-1000, Dosaka EM, Japan). Sections were mounted on aminopropylsilane (APS)-coated slide glasses, dried, stained with cresyl violet and imaged by a digital scanner.

1.3 Results

1.3.1 Behavioral effects of FLV

1.3.1.1 Residence time in naïve chicks (Experiment 1)

The mean ***residence time*** was shorter in {0.5, ×1.2} than that recorded in {2.0, ×1.1} irrespective of the food amount (1 grain or 2 grains) (Figure I-4A, B). Without interval increments, chicks left the *ON* feeder after the food supply was completed (vertical dashed line, {2.0, ×1.0}) or immediately after the first or second grain ({14.0, ×1.0}). Statistical analyses were thus performed for the other 4 groups of chicks, namely {0.5, ×1.2} and {2.0, ×1.1} for 1 and 2 grains per delivery. Based on mean residence time, AICs (Akaike Information Criteria) were compared among the 4 models in which the variables of *pattern* ({0.5, ×1.2} or {2.0, ×1.1}) and food amount (1 or 2 grains) were considered (Table I-1). The model with the *pattern* variable was chosen for its smallest AIC (72.95), and the model with the second smallest AIC (73.44) contained both *pattern* and *grain* variables, although the coefficient of *grain* (as indicated in parentheses) could contain 0 value at considerably high probability (p>0.05), thus the variable *grain* was not supposed to be significant. . The null model gave rise to the 3rd smallest AIC (86.88), and the model with the variable grain yielded an even larger AIC (88.12), indicating that the variable *grain* should not be considered. The present results are therefore compatible with one prediction of the optimal patch-use model (Figure I-3A), that the chicks leave earlier in the higher increment rate (×1.2 rather than ×1.1). However, the results did not match with another prediction (Figure I-3B), that chicks leave earlier in the larger gain rate (2 grains rather than 1 grain). In accordance with mean ***residence time*** (Figure I-4B), the mean ***leave-point interval*** (Figure I-4C) was

shorter in {0.5, ×1.2} than in {2.0, ×1.1} irrespective of the food amount.

The mean ***travel time*** (or the time spent in the *OFF* feeder) was also compared with the respective ***waiting time*** (or the time to the next delivery at the *ON* feeder) (Figure I-4D); symbols denote mean values in individuals. In both 1 grain and 2 grain trials, the ***waiting time*** tended to be longer than the ***travel time***, even though the more profitable option (the food available by revisit after a travel time) was temporally more proximate. Chicks waited longer for the next grain particularly in the condition of {0.5, ×1.2}. Most probably, chicks behaved retrospectively based on the time after the last grain was delivered, rather than based on prospective food gains.

1.3.1.2 Effects of FLV on residence time (Experiment 2)

To examine if the enhancement of endogenous 5-HT level could acutely or chronically affect patch-use behavior, we examined FLV effects for 3 successive days. A FLV solution (or vehicle) was injected (i.p.) 1 hour before the test of each day. We found that FLV elongated the mean ***residence time*** in both conditions acutely from the first day, but only at a high dose (20 mg/kgBW) (Figure I-5A). At a low dose (10 mg), however, significant effects were not found. We did not examine higher doses (40 mg or higher), because pilot trials revealed that higher FLV doses often caused chicks to sleep. In the following experiments, we therefore examined the effects of 20 mg FLV. Based on residence time, AICs were compared among the 4 models in which the variables of *drug* (vehicle, 10 or 20 mg/kg BW) and *day* (10–12) were considered (Tables I-2 and 3). Of these models, the full model yielded the smallest AIC (375.9 and 630.1 for the {0.5, ×1.2} and {2.0, ×1.1} conditions, respectively), and the coefficients of both variables (*drug* and *day*) included 0 value at probability $p<0.05$, thus the *drug* effect was

supposed to be significant. On the other hand, the models composed only with the *day* variables yielded a larger AIC (380.3 and 633.3, respectively), and AICs of the null models were even larger (394.7 and 645.0, respectively); significant drug effects were thus confirmed in both conditions of {0.5, ×1.2} and {2.0, ×1.1}.

1.3.1.3 Effects of FLV on inter-temporal choices (Experiment 3)

FLV suppressed choice impulsiveness (Figure I-5B), as the number of choices of the large reward (***LL***) were higher in the 20 mg group than in the vehicle control group. Based on the number of choices of the large reward, AICs were compared between the two models in which the variable *drug* (vehicle or 20 mg) was included or not (Table I-S1). The *drug* model had an AIC (46.74) that was smaller than the null model (49.09), and the *drug* coefficient included 0 value at $p<0.05$, thus the drug effect was supposed to be significant.

1.3.1.4 Effects of FLV on work efforts in shuttles (Experiment 4)

FLV decreased running distance and running velocity, but the number of visits, total residence time and residence time per visit were not influenced (Figure I-5C). For each parameter, AICs were compared among the 4 models in which the variables *drug* (vehicle or 20 mg/kg BW) and *day* (9–11) were considered (Tables I-S2 to S6). For *total residence time*, the null model was chosen for its smallest AIC. For *residence time per visit*, the model with the *day* variable was chosen. On the other hand, for *running distance* and *velocity*, full models were chosen with significant coefficients for the *drug* variable. For the *number of visits*, the full model was chosen. Taking all of these estimations into account, it is concluded that FLV suppressed the *total running distance*

by mainly slowing down the *velocity*, but the decision to leave the uncued food patches was not influenced, in a clear contrast to FLV effects on *residence time* at the diminishing patch (Experiment 2).

1.3.1.5 Effects of FLV on distress calls (Experiment 5)

FLV suppressed the number of distress calls, suggesting a general soothing effect. Two-way ANOVAs revealed significant effects on ***Δ distress calls*** (Figure I-5D) of dose (F_{dose} (2, 30) = 15.11, $p < 0.0001$), but not of day of experiment (F_{day} (1, 30) = 3.47, $p > 0.05$) without interaction ($F_{dose\ x\ day}$(2, 30) = 0.27, $p > 0.05$).

1.3.2 Pharmacological effects of FLV on 5-HT and DA in the striatum

1.3.2.1 Location of the microdialysis probes

Histological reconstruction of tracks revealed that the microdialysis probes were located in the mSt and surrounding tissues in all of the chicks studied (Figure I-6). These locations extended over the core and shell regions of the nucleus accumbens (Bálint & Csillag 2007, Bálint et al. 2011). In some chicks, the stained track was found in the lateral part of the bed nucleus of the stria terminalis and the ventral pallidum sub-regions of the basal ganglia ventromedial to the mSt (Reiner et al. 2004). These regions in the basal ganglia are densely innervated by 5-HT positive fibers (Metzger et al. 2002).

1.3.2.2 Microdialysis and systemic application of FLV (Experiment 6)

Intra-peritoneal injection of FLV (20 mg/kg BW) elevated concentrations of both 5-HT and DA in the perfusate. Samples were collected from chicks housed in an

illuminated chamber not equipped with feeders. Notice that the subject chicks did not forage food, nor did they actively walk around. Figure I-7Aa and Ac indicate the time-course of relative concentrations measured as % of the reference level (sample #0 indicated by an arrowhead). AUCs did not significantly differ between groups (Figure I-7Ab and Ad); $U = 17$, $p > 0.05$ for 5-HT; $U = 13$, $p > 0.05$ for DA (Mann–Whitney's U-test, $n_1 = n_2 = 6$). After the injection (samples #1 to #4), on the other hand, significant differences were found; $U = 0$, $p < 0.005$ for 5-HT; $U = 0$, $p < 0.005$ for DA. It is thus suggested that FLV could have an inhibitory effect on 5-HT re-uptake, but the effect was not specific, as the DA level was also elevated. The injected FLV could indirectly increase the DA level via unspecified pathways, or otherwise FLV (or the elevated 5-HT) directly enhanced DA release (or re-uptake mechanisms) in the striatum. We therefore conducted a reverse dialysis experiment, in which FLV was applied to the perfusing aCSF solution.

1.3.2.3. Reverse microdialysis and local application of FLV (Experiment 7)

Similar effects were found in the reverse microdialysis. FLV was added to the perfusate twice at 2 different doses (2 and 20 μM at sample #1 and #5, respectively), and considerable increases were found in 5-HT and DA after 20 μM (Figure I-7Ba and Bc). AUCs were compared among 3 phases of sampling, namely, samples #-4 to #-1 (aCSF), #1 to #4 (2 μM), and #5 to#8 (20 μM) by using Friedmann's tests ($k = 3$ blocks of treatments, $m = 8$ individuals); $S = 86$ ($p < 0.01$) for 5-HT, and $S = 78$ ($p < 0.01$) for DA, respectively. We therefore conclude that FLV elevated 5-HT and DA locally in the striatum.

1.4 Discussion

We designed the present experiments to mimic optimal patch-use behavior in the field. The residence time was dependent on the pattern of food supply (***pattern***) as expected, but not on the amount (***grain***) (Experiment 1; Figure I-4A, B and C). Furthermore, the ***travel time*** was generally shorter than the ***waiting time*** at the feeder (Figure I-4D); clearly, chicks did not consider the larger gratification obtainable by leaving the patch as an alternative option. We therefore agree with Stephens & Anderson (2001) that inter-temporal choice data do not give a good account for understanding patch-use behavior.

We expected to find that chicks with a systemic FLV application would leave the depleting feeder earlier than chicks with a vehicle control (Figure I-1), under the premise that the decision to leave a food patch is identical to the choice of the large/long-delay option in the inter-temporal choices. FLV actually suppressed choice impulsiveness (Experiment 3; Figure I-5B), confirming previous studies (see below for further discussion). However it is to be noticed that the effect of FLV on choices might be ascribed to altered sensitivity (1) to delay or (2) to amount of food reward; further experiments should be added in order to address to this issue. FLV elongated residence time, contrary to our initial expectation (Experiment 2; Figure I-5A). FLV also suppressed the running distance and velocity in the work effort test, as well as the distress calls induced by social isolation (Experiment 4 and 5; Figure I-5C and D), suggesting a spectrum of soothing effects. *In vivo* microdialysis revealed an increase in both 5-HT and DA by systemic and local application of FLV (Experiment 6 and -7; Figure I-6 and I-7), suggesting the possibility that the systemic FLV directly affected the

medial striatum. It is notable however that the dialysis experiments were performed without any tasks, whereas the behavioral effects of FLV were examined in chicks that were actively foraging in the maze. The increases in 5-HT and DA by FLV may appear differently under the behavioral conditions.

It is therefore concluded that (1) the present behavioral experiments only partially reproduced optimal patch-use behavior. (2) FLV suppressed impulsiveness in the inter-temporal choices, and (3) it also made chicks tolerant of the delay in the next food gained in the patch. Finally, (4) these behavioral effects of FLV might be mediated by enhanced action of either or both of 5-HT and DA in the medial striatum.

1.4.1 5-HTergic control of motor activities and residence time

FLV suppressed running distance and velocity in this study (Figure I-5C), suggesting that acutely enhanced 5-HT level decreases motor activities. In accordance with this finding, a previous report showed that a $5HT_{1}$ and $_{2}$ agonist (lysergic acid diethylamide) decreased the exploratory locomotor activity of rats (see review by Geyer (1995)). However, behavioral effects of 5-HT may be dependent on the context. SSRI induced hyperlocomotion in mice when tested in a novel environment, but not in a habituated environment (Brocco et al. 2002). In chicks, FLV effects should be examined in other contexts such as spontaneous motor activity in an open field without food.

On the other hand, FLV effects on residence time and the inter-temporal choices (Figure I-5A and B) may be explained differently, whereby 5-HT enhances the tolerance for delay of the forthcoming reward. The present findings are in concert with previous studies of behavioral pharmacology (Bizot et al. (1988, 1999) for acute effects of 5-HT uptake inhibitor, Bizot et al. (1999), Wogar et al. (1993), Mobini et al. (2000) for 5,7-

DHT infusion to dorsal raphe) and a recent neurophysiological analysis of raphe neurons (Miyazaki et al. 2011a; also see Miyazaki et al. (2011b) for a microdialysis study in the dorsal raphe nucleus). It must however be noticed that the acute effects of SSRIs have not been reproduced in inter-temporal choice tests using rats, in which subjects made choices by lever-pressing (Charrier & Thiébot 1996, Evenden & Ryan 1996). In these studies, the discrepancy with Bizot et al. (1988) has been ascribed to different task conditions.

Acute effects of SSRIs have also been questioned in a study using pigeons (Wolff & Leander 2002). They adopted behavioral titration for inter-temporal choices and examined a series of SSRIs (fluoxetine, citalopram and paroxetine; systemic application), but found only chronic effects that appeared after consecutive application for 17 days. The present results, on the other hand, revealed acute effects of FLV in chicks (Figure I-5B). The discrepancy may be ascribed to the different affinities of these SSRIs to the avian SERT (Larsen et al. 2004). While FLV strongly inhibits the chicken SERT (1.2 times in terms of K_i compared to human SERT), the SSRIs used Wolff & Leander (2002) had lower levels of inhibition (fluoxetine 5.6, citalopram 41 and paroxetine 41, respectively).

1.4.2 5-HT and interval timing (or retrospective / immediate sense of time)

Involvement of DA in the control of the internal clock has gained substantial experimental support (Meck et al. 2002, Coull et al. 2011 for review). Haloperidol, a D_2 receptor antagonist, is reported to delay the internal clock (MacDonald & Meck 2005). It is assumed that the corticostriatal circuit responsible for the internal clock is under speed-control by DA actions from the substantia nigra. However, significant action of 5-

HT on the timing mechanism is questioned. Ho et al. (1996) review recent studies of 5-HT actions on interval timing. It is notable that 5-HT depletion by 5,7-DHT application increases the Weber fraction of the timing behavior. However, they argue that 5-HT does not change the speed of the internal clock, but is involved in behavioral switching. Ho et al. (1996) actually report that systemic FLV does not influence indices of behavioral timing tested by a fixed-interval peak procedure.

It might be argued that the FLV effect on residence time (Figure I-5A) is explained by its action on interval timing, particularly through the unexpected increase in DA level by FLV (Figure I-7). However, the enhanced DA level should shorten the residence time by over-estimation of the perception of time, if its action is similar to amphetamine (Coull et al. 2011). It is thus not plausible that the observed effects are due to the accompanying DA increase. In conclusion, we may suppose that the perception of interval timing was not influenced by FLV in the present study.

1.4.3 DA release through 5-HT enhanced by FLV

Systemic application of FLV increased not only the 5-HT but also the DA level in the striatum (Figure I-7A), which was reproduced in the reverse dialysis experiment (Figure I-7B), indicating that FLV increased DA locally in the striatum. As reviewed by Navailles & Deurwaedere (2011), it is possible that the enhanced 5-HT could directly act on the dopaminergic terminals, making the DA transporter (DAT) reverse the direction of dopamine release. A similar mechanism may underlie the DA increase by FLV found in the present study. Alternatively, it is possible that FLV directly acted on the DAT and inhibited the DA uptake. Although data are not available on the avian DAT, a biochemical study has revealed that binding of FLV to human DAT is negligibly small

(Owens et al. 2001). The cellular mechanisms underlying the DA release by FLV need further investigation.

Co-release of 5-HT and DA by a 5-HT reuptake blocker (alaproclate) has already been reported in an *in vivo* experiment (Yadid et al. 1994) and also in *in vitro* preparations of striatum using an SSRI (fluoxetine, Zhou et al. 2005), but the functional significance remains unknown. Further detailed experiments are needed to reveal the functional significance of the cross talks between 5-HT and DA.

1.5 Figures

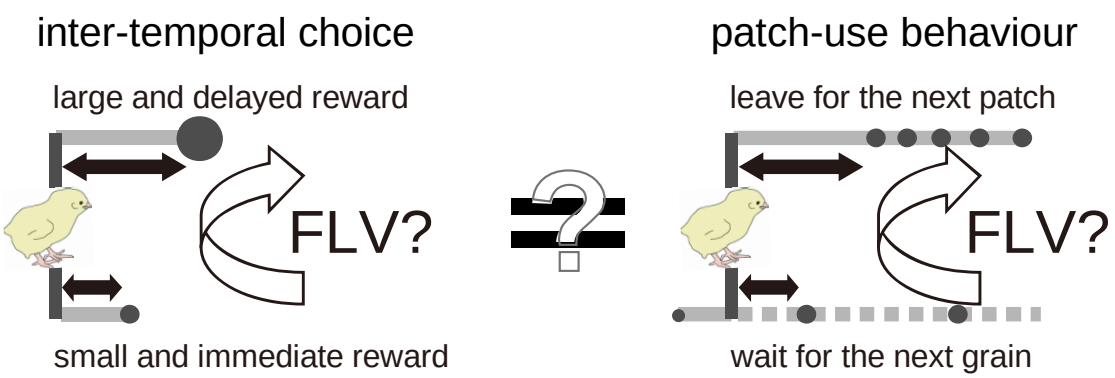


Figure I-1. Predicted effects of the fluvoxamine (FLV) on inter-temporal choice and patch-use behavior.

If the common self-control mechanism underlies the inter-temporal choice and the patch-use behavior, the FLV-treated chicks will leave a depleting patch earlier (or, a shorter residence time; right diagram) to gain food from the next patch. This prediction comes from the assumed effect of FLV on the inter-temporal choice (left diagram), suggesting that the FLV-treated chicks will prefer a large and delayed reward to a small and immediate reward.

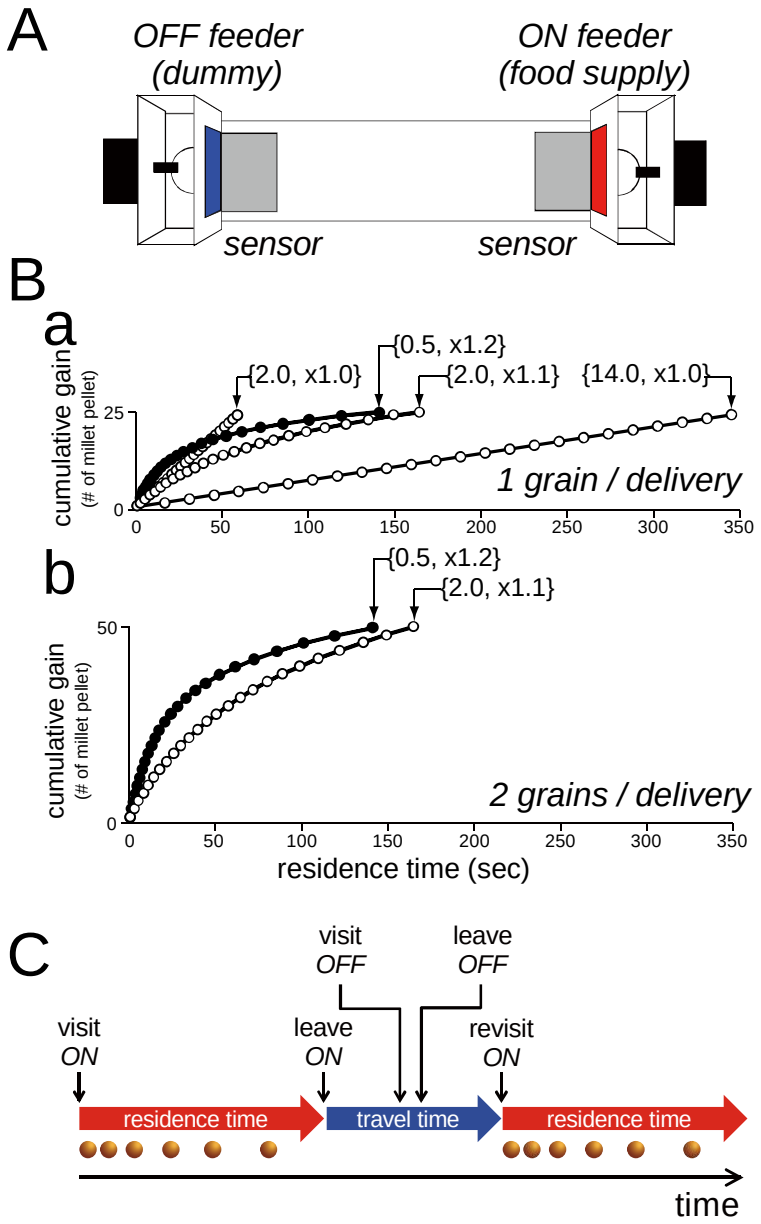


Figure I-2. Apparatus and timing of food delivery for the study of patch-use behaviors. (A) Schematic illustration of the apparatus. An I-shaped maze was composed of two feeders; *ON* feeder (red in this figure) served as a food patch, and *OFF* feeder (blue) did not supply food. As the chick arrives, the *ON* feeder starts to deliver food (1 or 2 grains of millet for each delivery) in a programmed manner as shown in (B). The *OFF* feeder similarly responds to the chick and terminates the food delivery at the *ON* feeder on the opposite side, but food is not delivered. (B) Cumulative gain (number of

grains of millet) is plotted against the residence time at the *ON* feeder. Figures in parentheses {α, xβ} indicate the initial interval α (sec), and the increment rate β. Four patterns of depletion were programmed up to a total of 25 (a) or 50 (b) grains. Two other patterns of a constant rate (β = 1.0) were also examined at short (2.0 sec) or long (14.0 sec) intervals. (C) Time course of food deliveries and chick's behavior. Each ball represents delivery of a grain of millet; note the gradually increasing intervals. *ON* and *OFF* denotes the *ON* feeder and the *OFF* feeder, respectively. Leaving the *ON* feeder stopped the food deliveries, and visiting the opposite *OFF* feeder reset the interval of the *ON* feeder to the minimum value (0.5 sec or 2.0 sec).

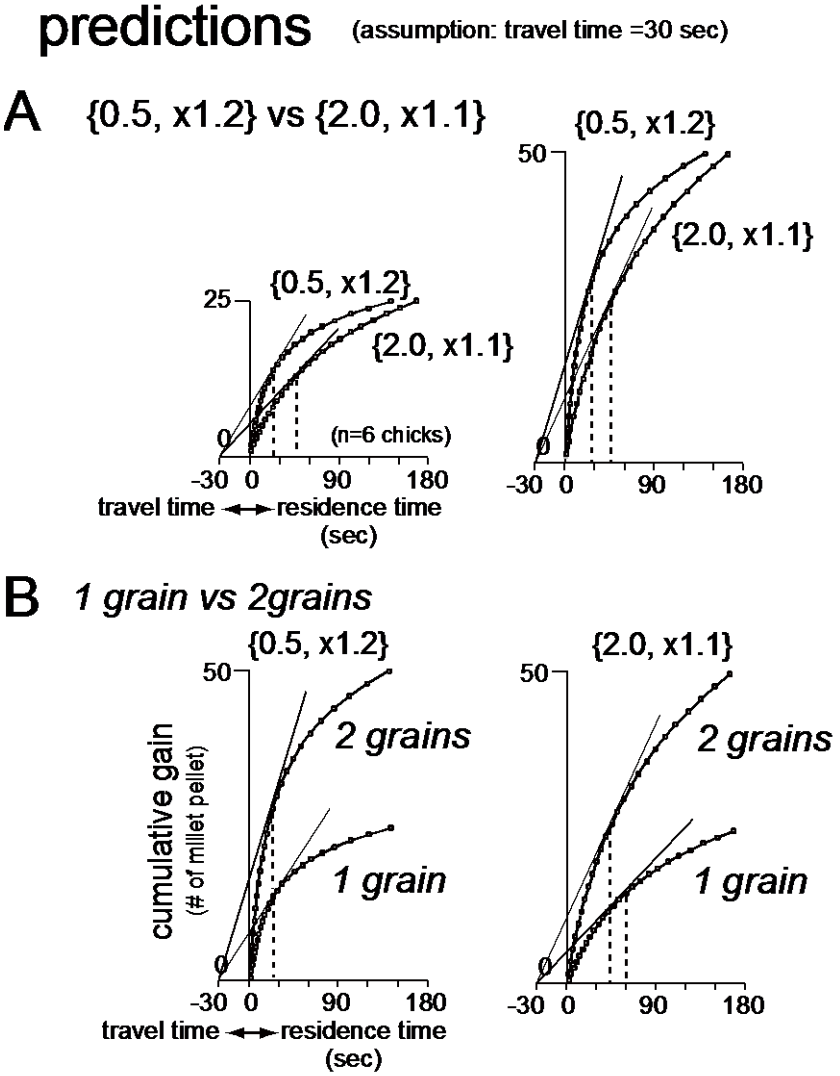


Figure I-3. Patch-use behavior and inter-temporal choice. If chicks behave optimally, we will find that the residence time is shorter in {0.5, ×1.2} than that in {2.0, ×1.1}(A), and shorter in 2 grains than in 1 grain (B), depending on the travel time that is assumed to be 30 sec in these graphs.

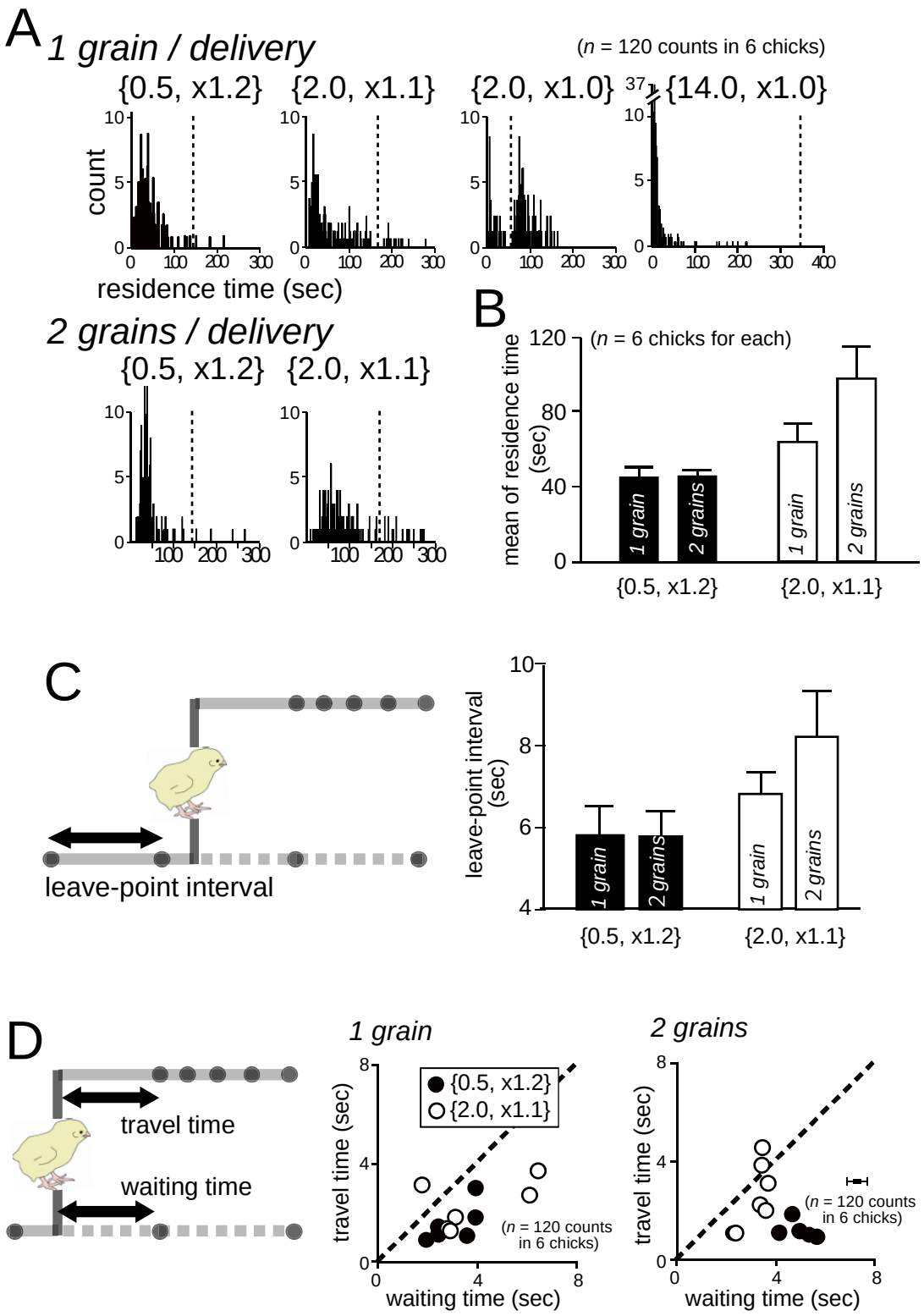


Figure I-4. Behaviors in depleting food patches (Experiment 1). (A) Residence time at

the food patch distributed with large variance. Histograms of residence time under different conditions are shown. Residence time was measured in 20 consecutive counts for each chick, and the total 120 counts (=20 × 6 chicks) were merged; data obtained on the last day (day 12) are shown. Vertical dashed lines indicate the terminal point at which the last grain was supplied. (B) Mean residence times of the four patterns are compared; error bars indicate S.E.M. (C) Leave-point interval. (D) Travel time (time until the next visit to the *ON* feeder) is plotted against waiting time (time to the next grain the chick could have gained if it stayed at the feeder); symbols represent individuals.

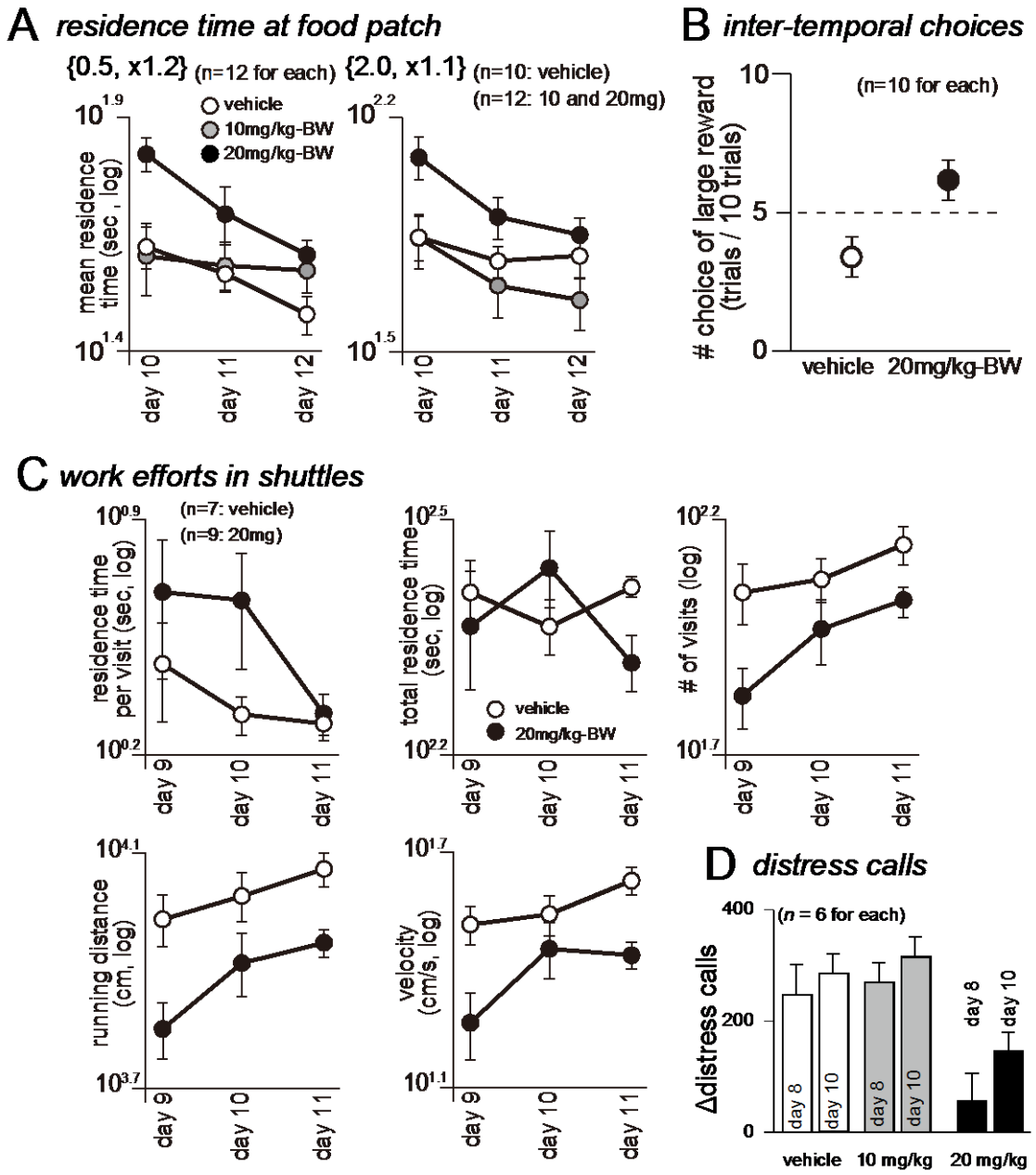


Figure I-5. Behavioral effects of FLV (fluvoxamine) on residence time (A), inter-temporal choices (B), work efforts (C), and distress calls (D). (A) Systemic injection of FLV resulted in a longer residence time. (B) FLV suppressed impulsiveness in the inter-temporal choices, but the difference was only suggestive (see text for statistics). (C) FLV decreased running distance and running velocity, but residence time per visit, total

residence time and number of visits were not influenced. Means (±S.E.M.s) are plotted for the day of experiment. (D) Increase in the number of distress calls (per 3 min) elicited by social isolation.

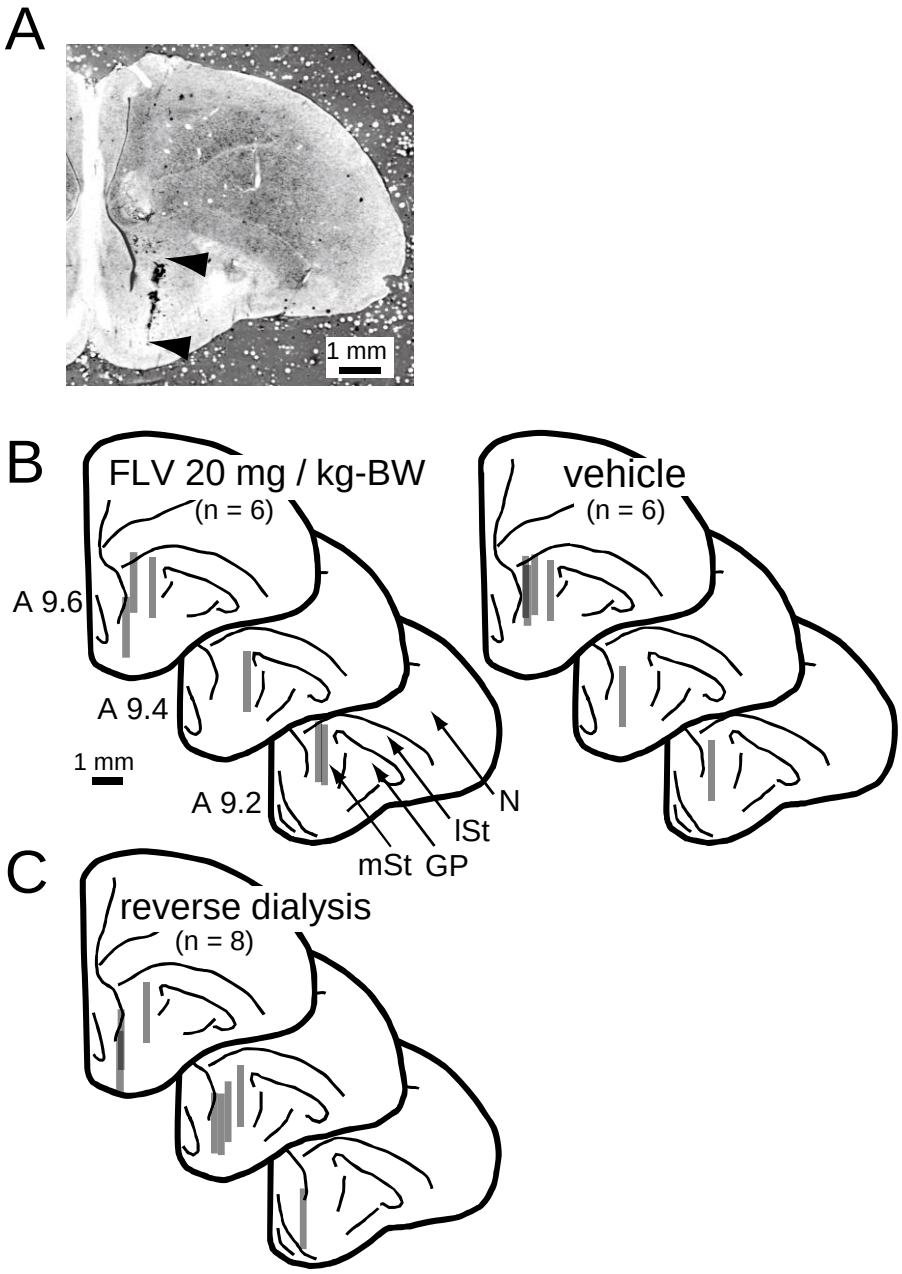


Figure I-6. Location of microdialysis probes in the striatum. (A) Representative photomicrograph of a track on a Nissl-stained section. Arrow heads point to dorsal and ventral edge of the probe stained by post-experimental injection of dye (0.1% pontamine sky blue). (B, C) Superimposed locations of probes are shown on frontal sections of telencephalon. N: nidopallium; lSt: lateral striatum; GP: globus pallidus; mSt: medial striatum.

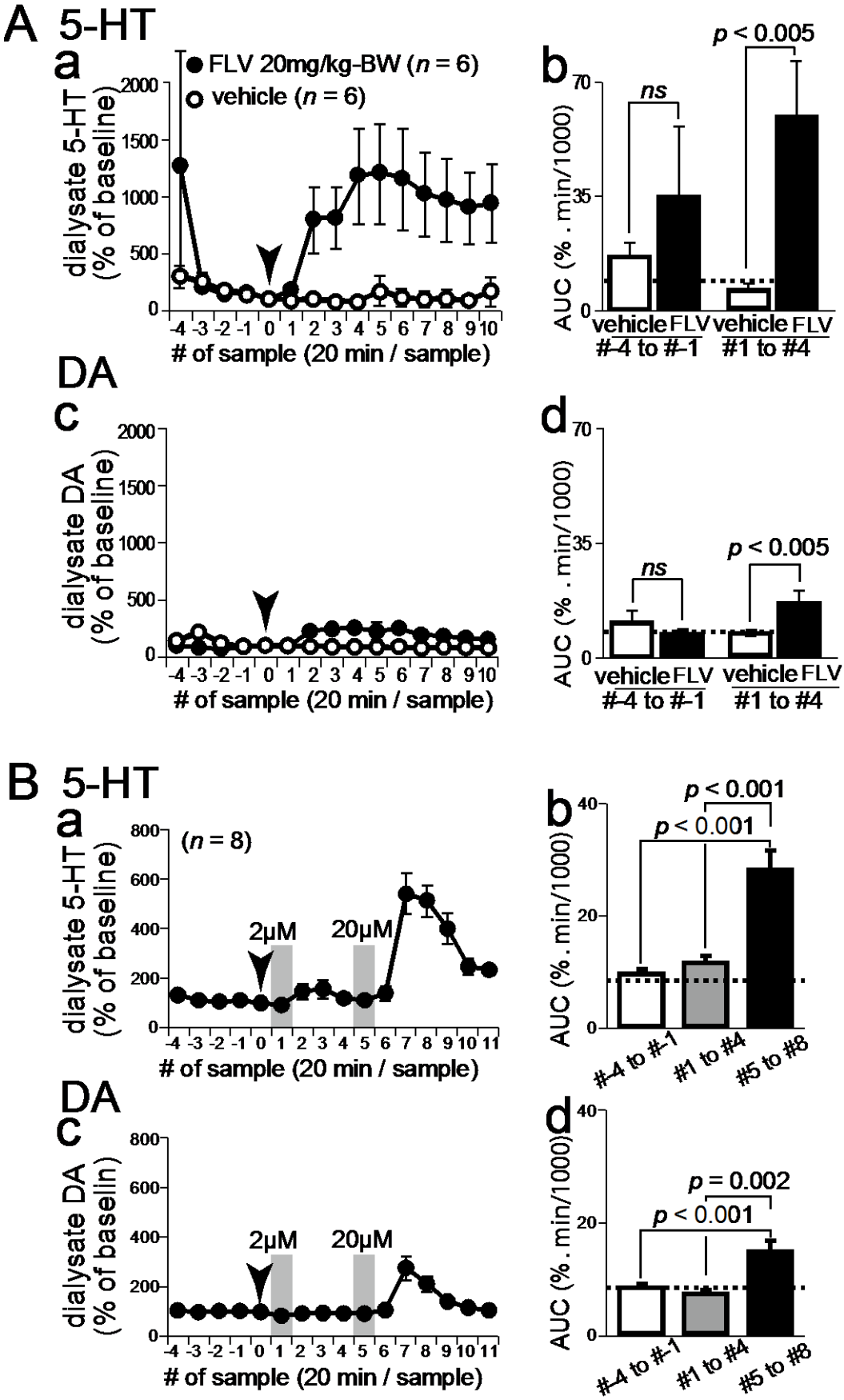

A 5-HT
a
FLV 20mg/kg-BW (n = 6)
vehicle (n = 6)
dialysate 5-HT (% of baseline)
# of sample (20 min / sample)
b
p < 0.005
ns
AUC (% . min/1000)
vehicle FLV vehicle FLV
#-4 to #-1 #1 to #4
DA
c
dialysate DA (% of baseline)
# of sample (20 min / sample)
d
ns
p < 0.005
AUC (% . min/1000)
vehicle FLV vehicle FLV
#-4 to #-1 #1 to #4
B 5-HT
a
(n = 8)
2μM 20μM
dialysate 5-HT (% of baseline)
# of sample (20 min / sample)
b
p < 0.001
p < 0.001
AUC (% . min/1000)
#-4 to #-1 #1 to #4 #5 to #8
DA
c
2μM 20μM
dialysate DA (% of baselin)
# of sample (20 min / sample)
d
p = 0.002
p < 0.001
AUC (% . min/1000)
#-4 to #-1 #1 to #4 #5 to #8

Figure I-7. Pharmacological effects of FLV on 5-HT and DA in the striatum. (A) Systemic injection of FLV (20 mg/kg BW) elevated the level of (a) 5-HT and (c) DA. Values are expressed as percent change relative to baseline (sample #0) as mean ± S.E.M. FLV (or vehicle) was intra-peritoneally injected at the beginning of the sample period #1 (arrowhead). The percent concentrations in 4 consecutive samples (namely 80 min) were summated to yield the area under curves (AUC) in b and d (mean ± S.E.M.); horizontal dashed lines indicate the baseline. AUCs were significantly different between FLV and vehicle only after the injection. (B) Local application of FLV through the probe revealed similar effects on extracellular 5-HT DA, suggesting direct action in the striatum. Time course and AUC of extracellular 5-HT and DA levels are similarly shown. AUCs were compared among three different phases of samples; samples #-4 to #-1 (aCSF), #1 to #4 (2 μM), #5 to #8 for (20μM), and significant differences were found among these phases. Note also that the effects on DA were reproduced.

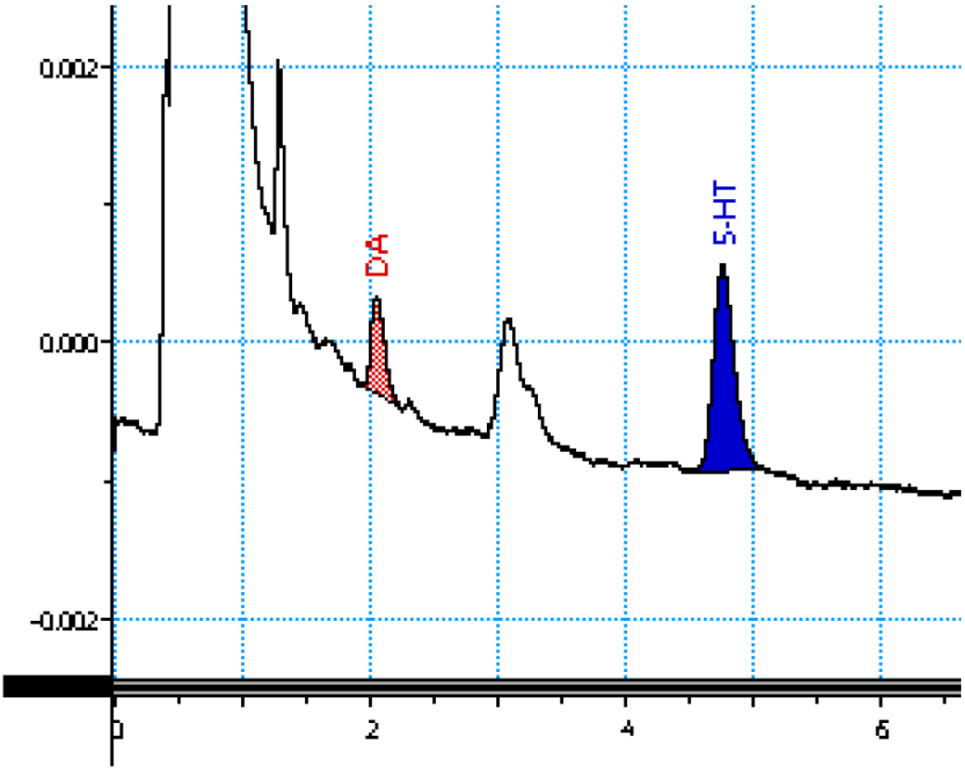


Figure I-S1. HPLC chromatogram (Experiment 6,-7). A typical example of HPLC chromatogram showing the clear separation of DA (dopamine) and 5-HT; plot against the elution time (X-axis, min). The coloured areas in red (DA) and blue (5-HT) indicate the AUC (area under curve; arbitrary unit) that represents the respective concentration in the perfusate.

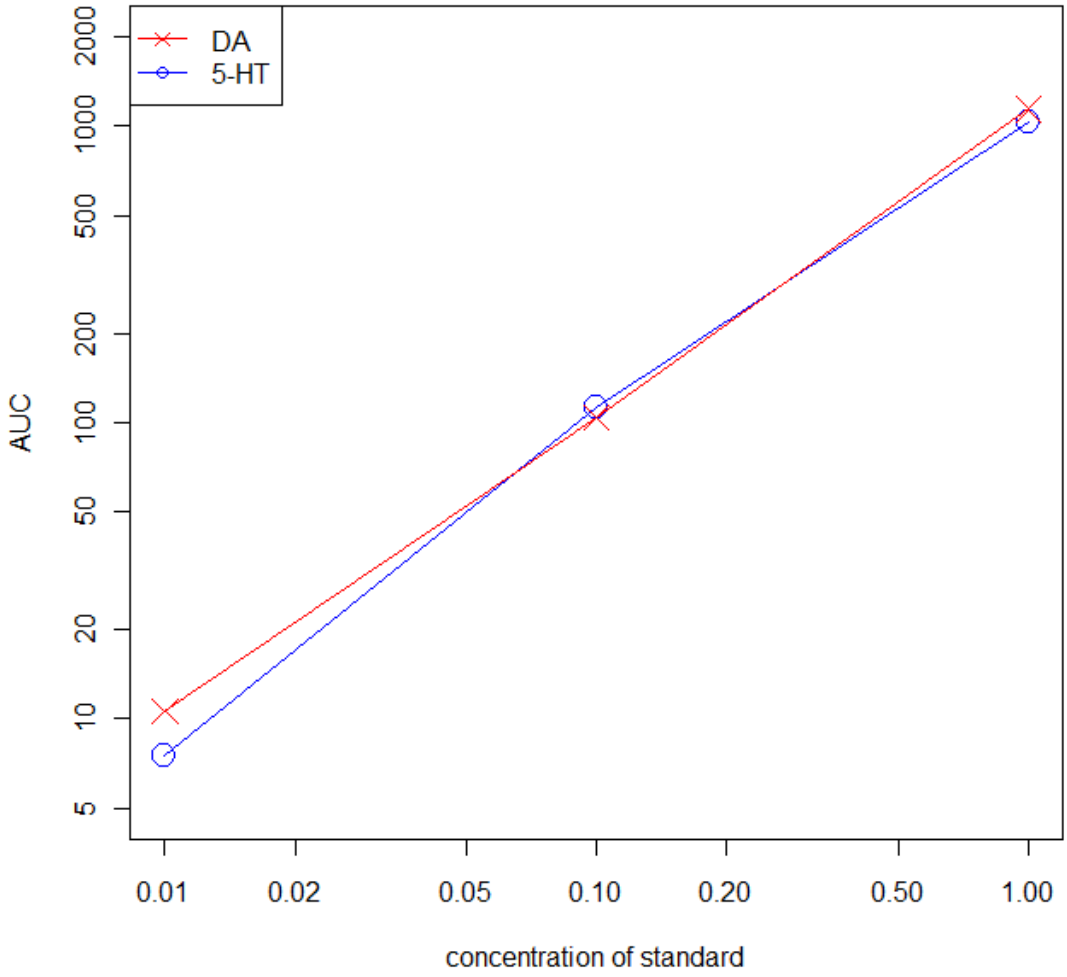


Figure I-S2. Standard curves of AUC (area under the curve; min x nA) plotted against the concentration of test solution that contained red (DA) and blue (5-HT) at 0.01, 0.1 and 1 ng/ml. Linear relationship was maintained in this range of concentration, which covered the range of data obtained in this study.

1.6 Tables

Table I-1. The *condition* model yielded the smallest AIC. AICs (Akaike Information Criteria) and estimated coefficients of variables were calculated for 4 models designed for mean of residence time in Experiment 1. The models are sorted in ascending order of AIC. The chosen model [α_0, α_1] with the smallest AIC indicates that *pattern* alone was significant, whereas the number of *grain* was not. Coefficients in parentheses represents that the 95% confidence interval (CI) of the estimate included 0. Most-likely-fitting formulas are indicated in this and the following tables.

models	AIC	estimated coefficients of variables		
		α_0 (*intercept*)	α_1 (*pattern*)	α_2 (*grain*)
1 (α_0, α_1)	72.95	3.7629	0.6190	-
2 $(\alpha_0, \alpha_1, \alpha_2)$	73.44	3.5270	0.6182	(0.1577)
3 (α_0)	86.88	4.07259	-	-
4 (α_0, α_2)	88.12	3.8376	-	(0.1567)

Table I-2. The *drug-day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for residence time ({0.5, x1.2} condition) in Experiment 1. The chosen model [β_0, β_1, β_2] indicates that both *drug* and *day* had significant effects.

models	AIC	estimated coefficients of variables		
		β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1 (β_0, β_1, β_2)	375.9	3.743069	0.024441	-0.152728
2 (β_0, β_2)	380.3	3.98778	-	-0.15307
3 (β_0, β_1)	390.8	3.65522	0.02640	-
4 (β_0)	394.7	3.92180	-	-

Table I-3. The *drug-day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for residence time ({2.0, x1.1} condition) in Experiment 1. The chosen model [β_0, β_1, β_2] indicates that both *drug* and *day* had significant effects.

models	AIC	estimated coefficients of variables		
		β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1 (β_0, β_1, β_2)	630.1	4.09510	0.03644	-0.20209
2 (β_0, β_2)	633.3	4.47996	-	-0.20173
3 (β_0, β_1)	641.9	4.04899	0.03772	-
4 (β_0)	645	4.4475	-	-

Table I-S1. The *drug* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 2 models designed for number of choice of large reward in Experiment 3. The chosen model [β_0, β_1] indicates that *drug* had significant effects. The 95% confidence interval of the estimated coefficient of the *drug* variable included 0 at a low probability (p = 0.0312), thus was supposed reliable.

models	AIC	estimated coefficients of variables	
		β_0 (*intercept*)	β_1 (*drug*)
1 (β_0, β_1)	46.74	-0.77334	0.05499
2 (β_0)	49.09	-0.2256	-

Table I-S2. The *day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for residence time per visit in Experiment 4. The chosen model [β_0, β_2] indicates that *day* alone had significant effects.

models	AIC	estimated coefficients of variables		
		β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1 (β_0, β_2)	250	5.0418	-	-0.3371
2 (β_0, β_1, β_2)	250.3	4.42556	(0.05505)	-0.33827
3 (β_0)	252.6	5.0258	-	-
4 (β_0, β_1)	252.9	4.41041	(0.05499)	-

Table I-S3. The *null* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for total residence time in Experiment 4. The chosen [β_0] model indicates that neither *drug* nor *day* had significant effects.

models	AIC	estimated coefficients of variables		
		β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1 (β_0)	1851	9.0412		
2 (β_0, β_2)	1852	9.04159	-	(-0.07504)
3 (β_0, β_1)	1852	8.86247	(0.01590)	-
3 $(\beta_0, \beta_1, \beta_2)$	1853	8.86263	(0.01591)	(-0.07507)

Table I-S4. The *drug-day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for running distance in Experiment 4. The chosen [β_0, β_1, β_2] model indicates that both *drug* and *day* had significant effects, although the effect of *drug* was not reliable.

models		AIC	estimated coefficients of variables		
			β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1	(β_0, β_1, β_2)	227.1	4.48989	(-0.03083)	0.21030
2	(β_0, β_2)	228.3	4.14643	-	0.20896
3	(β_0, β_1)	235	4.51214	(-0.03192)	-
4	(β_0)	235.8	4.1573	-	-

Table I-S5. The *drug-day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for running distance in Experiment 4. The chosen [β_0, β_1, β_2] model indicates that both *drug* and *day* had significant effects.

models		AIC	estimated coefficients of variables		
			β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1	$(\beta_0, \beta_1, \beta_2)$	4966	9.04361	-0.02881	0.16553
2	(β_0, β_2)	4967	8.71956	-	0.16549
3	(β_0, β_1)	4973	9.04392	-0.02883	-
4	(β_0)	4974	8.7197	-	-

Table I-S6. The *drug-day* model yielded the smallest AIC. AICs and estimated coefficients of variables were calculated for 4 models designed for velocity in Experiment 4. The chosen [β_0, β_1, β_2] model indicates that both *drug* and *day* had significant effects.

models		AIC	estimated coefficients of variables		
			β_0 (*intercept*)	β_1 (*drug*)	β_2 (*day*)
1	(β_0, β_1, β_2)	106	3.272511	-0.020937	0.151861
2	(β_0, β_2)	112.1	3.04252	-	0.14968
3	(β_0, β_1)	123.1	3.536127	-0.019376	-
4	(β_0)	127.4	3.29227	-	-

CHAPTER II

Social facilitation of foraging effort

in domestic chick

2.1 Introduction

Social facilitation, which results in an enhancement of behavioral performance or an increase in work investment when an individual is in the presence of one or more conspecifics, has been widely reported in a variety of animals including humans (Zajonc 1965 for a review), *e.g.*, ants building a nest (Chen 1937), cockroaches running in mazes for food (Gates & Allee 1933), hyenas in drinking behavior (Glickman et al 1997) and humans engaged in physical work (Triplett 1898) or mental work (Allport 1920), leading to recent report on physiological characteristics of the facilitation in cardiovascular responses (Blascovich et al. 1999). While many diverse taxa exhibit socially facilitated behavior, surprisingly little is known about the ecological contexts in which social facilitation occurs.

From an ecological standpoint, social interference by conspecifics is considered an important factor in influencing an individual's foraging strategies. Classical foraging theory typically focuses on the perspective of a single forager (Charnov 1976b); however this neglects the overall interactions that occur in group-living animals. A more recent perspective, coined the "Social Foraging Theory", suggests that not only individual decision-making, but also conspecific behavior, influences the outcome of an individual's foraging success (Giraldeau & Caraco 2000). For example, Parasitic Jaegers (*Stercorarius parasiticus*) will forage in larger groups when engaging in risky behavior such as attacking and steeling fish from Common Terns (*Sterna hirundo*), even though they could gain more food by foraging in smaller groups or by themselves (Bélisle 1998). Bélisle (1998) argued that the risk of starving must also be included in foraging theory in addition to such things as net/gross intake rate or efficiency, since

group formation is expected to reduce variance of the food-encounter rate.

In behavioral studies using chicks, Tolman & Wilson (1965) reported that paired chicks consumed a larger amount of food than did isolated chicks only when the chicks had been deprived of food. However, their study failed to show conclusive results on the effects of social facilitation on rates of pecking (Tolman 1967). Tolman & Wilson (1965) also did not control the amount of food chicks could consume, thus it was uncertain whether the increased consumption was a result of the amount of food available rather than mere social facilitation. In our study, we tried to know whether social facilitation could improve individual pay-off (*i.e.*, rate of net gain = [benefit – work cost] / time), or otherwise other currency (*e.g.*, low probability of starvation; Caraco *et al.* 1980) should be considered.

In the present study, we examined the influence of social facilitation in 1-2 week-old domestic chicks (*Gallus domesticus*). By strictly controlling the amount of food delivered, we examined whether foraging competition (*i.e.*, reduction of gain by interference of other individuals) may socially facilitate an increase in the amount of foraging efforts in chicks. Chicks provide a unique opportunity for studying the neuroscience of decision-making in relation to behavioral ecology and economics (Matsushima *et al.* 2003, 2008 for reviews), because we can quantitatively control feeding conditions and potential energy budgets in experiments. Furthermore, chicks are precocial animals that begin to forage independently as soon as they hatch, and so individual development can be controlled as well. In our study, we investigated two foraging behaviors, running and pecking, in order to assess whether foraging efforts would increase under specific social conditions (i.e. social facilitation). Running to, or approaching food, and pecking at, or handling, food have already been shown to have

distinct neural substrates involved (ventral striatum/nucleus accumbens and arcopallium, respectively) (Matsushima *et al.* 2008). For example, lesions of the ventral striatum enhanced choices of small/immediate reward against large/distant alternative (Izawa *et al.* 2003, Aoki *et al.* 2006a). Similarly, lesions of the arcopallium caused chicks to choose the small/easy reward more frequently than the large/costly alternative (Aoki *et al.* 2006b). It is therefore possible that social facilitation can occur differently in these two aspects of foraging effort.

2.2 Materials and Methods

2.2.1 Subjects

All 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, chicks were sacrificed in carbon dioxide according to the guidelines.

A total of 99 male domestic chicks (*Gallus domesticus*, White Leghorn strains) were used. New hatchlings (post-hatch day 1: presumed hatching day) were obtained from a local supplier (Hokuren Central Hatchery, Iwamizawa, Hokkaido, Japan). Chicks were paired and housed in transparent plastic cages (15 x 28 x 12 cm) under white lighting (12L: 12D; light period starting at 08:00) and thermo-controlled at *ca*. 30°C. Pairs of chicks in the same cages were trained and tested in the same conditions.

Two types of food were given, grains of millet and chick mash food. The total amount of food per day was kept at a certain level so that (1) the body weight of chicks gradually increased and (2) the chicks actively consumed food during experiments. From post-hatch day 2, chicks were fed mash food. The amounts of mash food were 1 g (post-hatch days 2-5), 1.5 g (days 6 and 7), and 3 g (from day 8). From post-hatch day 3, grains of millet were added. The amounts of grains (per chick per day) were 1 g (day 3), 2 g (day 4), 3 g (day 5), 2.5 g (days 6 and 7), and 2 g (from day 8). Until day 3, all chicks were communally fed. From day 4, chicks were allocated to a communally fed condition or solitarily fed condition depending on experiments: varied among groups in experiment 1 and solitarily in experiments 2 and 3. In the groups of solitarily-fed

chicks, each individual was fed in a cage that was visually separated by a black plastic wall, so that chicks did not see the other chicks eating food; these chicks were communally housed except when the daily diet was given.

2.2.2 Apparatus

In Experiment 1, an I-shaped maze equipped with one lane (1-lane maze; 12 cm in width, 88 cm in length and 30 cm in height) was used (Figure II-1A). The maze was equipped with a pair of terminal feeders. Walls at the terminals were colored red (left) or blue (right). Each feeder supplied a grain of millet food at variable intervals. Plastic petri dishes (5.5 cm in diameter to a depth of 1.5 cm) were used as food trays, and the floor of each dish was covered with sponge.

In Experiment 2, an I-shaped maze equipped with two lanes (2-lane maze; 25 cm in width, 88 cm in length and 40 cm in height) was used (Figure II-1B). These 2 lanes had the same width as that of the 1-lane maze used in Experiment 1 but were separated by a transparent acryl board in one groups of chicks. In another group, in order to visually separate the two lanes, opaque white cardboard was attached to both sides of the acrylic board so that the chicks could not see each other. On each terminal feeder, two food trays were placed in adjacent positions over the separation board. Rectangle-shaped food trays (3 cm in width, 4 cm in length and 2 cm in depth) with sponge on the floor were use. In the shared feeder condition, a 4-cm-wide window was opened on each terminal end of the separation board, and the chicks in both lanes shared the food supplied to a food tray (6 cm in width, 4 cm in length and 2 cm in depth) placed at the center.

In Experiment 3, the same 2-lane maze as that in Experiment 2 was used after a

slight modification to the food trays (Figure II-1C). Square food trays (width and length of 3.6 cm depth of 1.8 cm) were used with two different types of floor coverage: one with sponge and the other with acrylic plates with 25 holes in the surface (aligned in 5 x 5, 4 mm in diameter). Four different types of acryl plates were used with different depths of the holes: 1.5 mm, 1.8 mm, 2.0 mm and 2.3 mm. Food trays were manually replaced, and a single replacement took *ca*. 10 sec.

In all experiments, in order to prevent chicks to associate the feeder sound with food reward, sounds of electric motors were replayed at variable intervals from instruments placed around the apparatus; the mean interval was set at 2.5 seconds, uniformly distributed from 1.5 to 3.5 seconds. The apparatus was placed in a dark room kept at *ca.* 25-30°C and illuminated by four 60 W white light bulbs placed above the runway and feeders. Timing of grain delivery and the noise sound were controlled by microrobots (RCX, LEGO Mindstorms). Behavior of the chicks was recorded by a video recorder (DCR-SR65, Sony, Japan) and color CCD cameras (250k pixels with NTSC output), and the recordings were stored for offline analysis.

2.2.3 Behavioral procedures

In Experiments 1 to 3, chicks were initially habituated to the experimental maze according to a common procedure for 2 successive days (pre 1 and pre 2) on post-hatch days 6-14. For habituation, paired chicks (housed in the same cages) were placed on the midway part of the maze in which some food (*ca.* 100 grains) was given in advance. After the chicks had consumed the food, feeders started to deliver grains by the same procedure as that used in each experiment. Two successive habituation sessions (*ca.* 20 min in total) were given per day for each of pre 1 and pre 2 except for Experiment 3, in

which one long session of habituation (*ca.* 20 min) was given instead. The experimental data were obtained after the habituation.

2.2.3.1 Experiment 1: effects of paired foraging on approaching food, an inter-group comparison in the 1-lane maze

Effects of paired foraging in the maze and the cage were examined in regards to subjects' distance and synchrony during running. Running distance was used to calculate the rate of approaching food at either end of the I-maze, where an increase in distance corresponds to increased running during the experimental condition.

"Paired in the maze" means that chicks foraged in pairs at the test, whereas "paired in the cage" means that the chicks foraged in pairs in the housing cage, in which main diet (mixture of mash food and millet) was supplied (Figure II-3A). Chicks were thus divided into four conditions: (1) paired in the maze / paired in the cage, (2) paired in the maze / single in the cage, (3) single in the maze / paired in the cage, (4) single in the maze / single in the cage.

After the habituation (pre 1 and pre 2), experiments were conducted for 5 consecutive days (days 1-5) and chicks received one test session per day. In each of the sessions, after a short habituation period in the maze (*i.e.*, 1 min after the chick had consumed all of the food available, namely, 20 grains / chick placed in advance in each feeder), each of the two feeders supplied one grain at a time with variable intervals (mean interval of 15 sec, uniformly distributed in a range of 10-20 sec). In a single session, the programmed food delivery continued 30 times and thus lasted for *ca.* 8 min. The chicks were then left in the maze for an additional 2 min after they had consumed all of the grains delivered on the food trays, and the test session of the day was

terminated.

In the I-shaped maze, the following 2 parameters were measured: (1) running distance (or how far the chick ran) and (2) synchrony index. Synchrony index was defined as the ratio of time in which both chicks stayed in the same side of the maze, shown as % of the total time recorded. The position of each chick's head was automatically and unequivocally given by computer-based video analysis, as either being placed on the red or the blue side of the maze. When both chicks were always in the same end, the synchrony index was 1.0 (in-phase synchrony). When, on the other hand, chicks were in opposite ends, the index was 0.0 (anti-phase synchrony). When both chicks moved in a random and independent fashion, the index would show a chance level of 0.5 (asynchrony).

2.2.3.2 Experiment 2: effects of paired foraging on approaching food, an inter-group comparison in the 2-lane maze

In order to differentiate food competition from social facilitation by a nearby conspecific, we removed the factor of food competition by separating paired chicks with either a transparent or an opaque partition down the middle of the maze's track, thus creating two separate lanes (Figure II-1B). After the habituation (*i.e.*, paired foraging in the maze on pre 1 and pre 2), similar to Experiment 1, test sessions were repeated 5 times, one session per day (days 1-5). Each of the two feeders supplied one grain at a time with variable intervals (mean interval of 15 sec, uniformly distributed in a range of 10-20 sec). In a single session, the programmed food delivery continued 30 times and thus lasted for *ca.* 8 min. The following two parameters were measured: (1) running distance and (2) synchrony index.

2.2.3.3 Effects of paired foraging on running distance, an intra-individual comparison

The immediate effect of paired foraging was examined in terms of running distance. A group of chicks (post-hatch days 12-14) were re-used after Experiments 1 and 2 (see above); chicks that had been solitary fed in both the maze and the cage were used. Immediately after each chick had been individually placed in the maze, both feeders delivered grain 10 times at variable intervals (mean interval of 15 sec, uniformly distributed in a range of 10-20 sec, 1 grain per delivery); this term is referred to as the *first "single" phase*. The companion chick was then introduced into the maze, and the feeders delivered twice as much grain (2 grains per delivery) 10 times; this term is referred to as the second *"paired" phase*. In the third phase, the companion chick was removed from the maze, and the subject chick received feeding another 10 times. Each phase lasted for *ca.* 3 min.

2.2.3.4 Experiment 3: effects of paired foraging on approaching and pecking at food, an inter-group comparison in the 2-lane maze

Effects of visual perception of the other chick on running (i.e., approaching food) and on food pecking (handling food) were examined in the 2-lane maze. The food tray difficulty (estimated on the basis of the number of pecks required for chicks to gain a certain amount of grain) was controlled by systematically changing the types of acryl plates used as the floor of the feeder (Figure II-1C). After habituation (*i.e.*, paired foraging in the maze on pre 1 and pre 2), similar to Experiments 1 and 2, test sessions were repeated 3 times, one session per day (days 1-3); chicks were left untested for 4 days between test days 1 and 2. Each of the two feeders supplied two grains at a time

with variable intervals (mean interval of 30 sec, uniformly distributed in a range of 20-40 sec).

In the tests, the variable feeder initially had a sponge floor. When the variable feeder had delivered 6 times (12 grains), the sponge was replaced by an acryl plate with 1.5-mm-deep holes. The 1.5 mm plate was subsequently replaced by 1.8, 2.0 and 2.3 mm plates when the variable feeder had delivered 12 grains for each plate. Four CCD cameras were set just above the feeder to record pecking behavior of the chicks were video recorded. The following four parameters were measured: (1) number of pecks, (2) number of gained grains, (3) running distance and (4) velocity. Velocity (cm/s) was measured in the runway except for the areas near the feeders (<10cm from the walls).

2.2.4 Data analysis

2.2.4.1 Recording and analyzing approaching behavior

Experiments were videotaped and coded later at rate of 30 frames per second using a Handycam recorder. The Handycam was located directly above the I-maze during testing, providing an aerial view of the subjects and apparatus. Chicks were individually marked by a rectangular piece of fluorescent-colored tape (Yamato Co., Ltd., Japan) affixed to their heads. The position of the fluorescent markers was analyzed by using Move-tr/2D 7.0 software (Library Co., Japan) and the trajectories and running distances were thus calculated.

2.2.4.2 Statistical analysis of generalized linear mixed model (GLMM)

The following five types of behavioral parameters were analyzed by using R for the platform of statistic calculation: running distance, synchrony index, number of

pecks, number of gained grains and velocity. See Supplementary Appendix for details.

2.3 Results

Once habituated, chicks began to actively run between the terminal feeders as soon as they were put in the maze, even without any visual cues. Furthermore, the runs were not in response to the timing of food delivery. Singly-tested chicks stopped running within *ca.* 1 min of the final delivery of food items. We therefore assumed that running distance of the runs represented "approaching effort" and pecks at the feeders represented "handling effort", rather than reflexive responses to food, and examined food-approaching behaviors in a series of experiments.

2.3.1 Experiment 1: Paired foraging increased running distance.

Paired chicks ran more than single chicks did. Figure II-2A shows representative running trajectories of single (top and 2nd records) and paired chicks (3rd and 4th records) at tests. Runs by single chicks were irregular on day 1 (top record), but they were more regular and more active on day 5 (2nd record). Runs by paired chicks were highly synchronized on day 1 (3rd record), but they were unsynchronized on day 5 (4th record). Superimposed trajectories (Figure II-2B), however, showed that the runs were not in response to the timing of food delivery.

Paired foraging in the maze, but not in the cage, increased running distance and synchrony index. A comparison of running by the four groups of chicks (n=10 in each groups) is shown in Figure II-3B. Based on running distance, AICs were calculated for each of the 8 models in which the variables of *day* (1 to 5), *maze* (paired or single) and *cage* (paired or single) were considered (See Table II-S1 of supplementary material for details.). Of these models, the *day-maze* model yielded the smallest AIC. The *day-maze-*

cage model yielded the same AIC, but the *cage* term was not reliable for its coefficient (p=0.197). Similarly, based on the synchrony index, AIC calculations revealed a facilitating effect of paired foraging in the maze, but not those of paired foraging in the cage (Table II-S2). We therefore concluded that the presence of other chicks and/or food competition among paired chicks could have caused the increase in approaching effort. It is not clear whether synchronization might be directly (and causally) linked to the increased effort.

2.3.2 Experiment 2: Visual perception of the other chick, but not food competition, excessively increased running distance.

To reveal the cause of the increased running distance, we separated the maze into two lanes by a transparent/opaque wall (see Figure II-1B), and we found that visual perception increased both running and synchrony indexes. Figure II-4 shows representative trajectories obtained from one pair in each group. When separated by an opaque wall, chicks in the 2-lane maze (therefore with no food competition) ran back and forth between feeders independently (top record in Figure II-4). When visually interacting via a transparent wall, chicks ran back and forth in high synchronization (second record). Direct foraging competition at shared feeders (see Figure II-1B, bottom) did not result in difference from when the transparent wall separating the feeders was present (compare second and bottom record in Figure II-4). It is notable that a high degree of synchrony was maintained until day 5, in contrast to the unsynchronized running seen in Experiment 1 (see Figure II-2A, fourth record, and Figure II-3B, bottom).

Based on the running distance and synchrony index (Figure II-5), AICs were

calculated for 8 models. As variables, *day* (1 to 5), *wall* (whether the wall was transparent or opaque), and *feeder* (whether the feeders were separated or shared) were considered (Table II-S3). For running distance, the *day-wall* model yielded the smallest AIC (16191). The *day-wall-feeder* model gave rise to the second-smallest AIC (16192), but the *feeder* term was not reliable for its coefficient (p= 0.2921). For synchrony index (Table II-S4), the *day-wall* model yielded the smallest AIC, but the *day* term was not reliable for its coefficient (p= 0.0844); thus, the result is different from the synchrony index in Experiment 1, in which the coefficient of the *day* was negative. We therefore tentatively conclude that the running distance and synchrony index are not linearly linked. Increased effort is not brought about by changes in synchronized running.

2.3.3 Pairing immediately increased running distance.

In both Experiments 1 and 2, difference in running distance between the groups appeared from day 1 of the experiment. In order to determine whether paired foraging could immediately increase the approaching effort, we examined running distances by an intra-individual comparison. As shown in a typical example (Figure II-6A), pairing immediately increased running. The average running distance for each condition (mean ± SEM, n=14) is shown in Figure II-6B. The Wilcoxon signed-rank test revealed a significant difference between paired phase and mean of the first and last phases (T = 2, p-value = 0.0003662).

Experiment 3: Pairing increased pecks without improved food gain.

In order to determine whether paired chicks also increased their handling effort (i.e. efforts to collect food), we examined rates of pecking for food by using a series of

trays that modified the difficulty for food collection (Figure II-1C). Paired chicks increased the number of pecks, particularly in the difficult food condition (in a range from 1.8 to 2.3mm) (Figure II-7A). The number of grains decreased in accordance with increased difficulty of food tray, but the number of grains was not different between the single and paired groups (Figure II-7B). Running distance was greater for paired chicks as found in Experiments 1 and 2, but the difference gradually diminished in the difficult food condition (Figure II-7C). However, the velocity at which paired chicks ran remained consistently higher than in the single chicks (Figure II-7D). The running distance of paired chicks decreased not because they ran slowly but because they stayed at the feeders for a longer time.

For all of the data shown in Figure II-7A-D, AICs were calculated for 5 models in which the variables of *feeder* (food tray difficulty, 5 steps ranging from sponge to 2.3 mm), *maze* (paired or single), and their *interaction* were considered (Tables II-S5 to S8). For the number of pecks, the *feeder-maze-interaction* model yielded the smallest AIC (Table II-S5), indicating that the effects of pairing emerged on handling effort only when the food was difficult to obtain. For the number of grains, on the other hand, the *maze* term was not included in the chosen model (Table II-S6), indicating that the paired and single chicks had similar gains. For running distance, the *feeder-maze-interaction* model was chosen (Table II-S7). The *interaction* term suggested that the difference between the paired and the single chicks were smaller for the more difficult food trays. For velocity, the *maze* model was chosen (Table II-S8).

2.4 Discussion

2.4.1 Ecological accounts of the excessive foraging efforts

In this study, we found that visual perception of other individuals, rather than direct foraging competition, increased foraging efforts for approaching food (running distance) as well as for handling food (number of pecks). Increased foraging efforts in this study appear to be a result of social facilitation. Zajonc (1965) argued that social facilitation affects "dominant responses", meaning that dominant (or well-developed) action patterns are most likely to be socially enhanced. Both running and pecking are well-developed behaviors in actively foraging domestic chicks. It should be noted, however, that chicks never exhibit running or pecking behavior when food is not available (data not shown), indicating that direct inter-individual interactions alone failed to cause social facilitation. It should also be noted that the term "social facilitation" is a psychological label, never specifying its functions in terms of economics / ecology. The idea of social facilitation is therefore not mutually exclusive with the idea of work investment under competition.

Our results suggest that currencies (or value functions) other than the food benefit are critical in social facilitation, in accordance with social foraging theory. According to Koops & Giraldeau (1996), starlings adopt foraging tactics that minimize the probability of energetic shortfall rather than maximize mean intake rate (or the benefit). Chicks may also make use of other individuals' information at food sites to avoid the risk of starvation, given that chicks have been shown to be risk-averse to food quantities (Kawamori & Matsushima 2010). However, much remains to be discussed about whether excessive running and pecking was really "inefficient", since we were

not able to calculate the energetic cost of running and pecking in terms of joules / calories. It is still possible that running and pecks are energetically very cheap and that the overt investments found in this study do not cause a negative energetic budget. Precise measurements of physical expenditures for running and pecking actions are needed.

Excessive foraging efforts found in this study might also lead to impulsive choices. Amita et al. (2010) reported that repeated experience of competitive foraging for a few days resulted in a higher level of choice impulsivity, though the chicks were solitarily tested in an inter-temporal choice paradigm. On the other hand, the impulsivity did not change instantaneously when the chicks were tested in competition (Amita and Matsushima in the accompanying paper); the increase in impulsivity was observed even when there was no actual food competition, similar to our results. It remains to be determined whether the visual perception of competitive individuals directly caused the impulsivity or whether the excessive work investments secondarily caused the impulsivity. Further studies are required.

2.4.2 Ecological accounts of synchronized running

The synchronized running observed in this study (Experiments 1 and 2) may be explained by "scramble kleptoparasitism" in behavioral ecology. Kleptoparasitism refers to parasitical exploitation of food that other foragers' efforts have made available (Giraldeau & Caraco 2000). Of several variations, scramble kleptoparasitism specifically refers to the simultaneous exploitation of a sharable resource by multiple competitors with little or no aggression. This foraging behavior has also been called "social facilitation" (Curio 1976). Barnard & Sibly (1981) regarded interactions

between kleptoparasitically foraging individuals as ‘producer/scrounger’ relationships, or a pair of alternative strategies.

It is possible that chicks running together acted as producer and scrounger, the one leading acted as a producer and the other following acted as a scrounger. In this study, we found that some individuals behaved predominantly as followers; *e.g.*, in the bottom record of Figure II-4, the purple-colored chick tended to follow the green-colored chick. However, the tactics were not always fixed, and chicks often changed their position in reference to the other. Foraging competition did not influence the synchrony of running (Experiment 2, Figure II-5, bottom), indicating that chicks did scramble kleptoparasitism due to some innate or developmental factors, rather than to immediate competition over food. In accordance with this, we often observed that a chick was attracted to its pair mate and stayed at the feeder, even though the subject chick had already gained a grain at that feeder (*e.g.*, bottom traces in Figure II-4). To reveal the direct cause of synchronization, more elaborate analysis of running synchrony is needed.

The synchronization may also be an adaptive response to predation risk. Hamilton (1971) points out that predation could lead to the evolution of gregarious behavior by considering that predators habitually approach from outside of the herd. Furthermore, gregariousness itself “dilutes” predation risk for any particular individuals (Foster & Treherne 1981). Highly synchronized runs observed in our study may have resulted from the gregarious instincts in chicks. It is therefore quite interesting to examine if chicks under predation pressure could run in even higher level of synchronization.

2.4.3 Implications for neural mechanisms of social facilitation

In the neuroscience of decision making, relevant brain regions and neurotransmitters / neuromodulators critical for "effort cost" (such as pressing a lever or climbing a mesh barrier to obtain food pellets) have been intensively explored (Walton et al. (2006), Floresco et al. (2008a)). Several brain regions are reported to cause a work cost aversion, meaning a bias away from the costly option (*e.g.*, climbing mesh barrier) to obtain a larger food; *i.e.*, the anterior cingulate cortex (Rudebeck *et al.* 2006a), the neural pathways between the anterior cingulate cortex and amygdala, and the amygdala itself (Floresco & Ghods-Sharifi 2007). Systemic administration of dopamine antagonists (Denk *et al.* 2005; Floresco *et al.* 2008b) also induced the same effect. Since the anterior cingulate cortex and amygdala are thought to be involved in social behaviors (Rudebeck *et al.* 2006b; Rosvold *et al.* 1954), these regions might play a critical role in social influences of behavior by conspecifics. However, no studies have so far integrated the neuroscience of economical decision making and the behavioral ecology of social foraging. Our next goal is therefore to clarify the neural mechanisms that underlie the social influences on work investments.

2.5 Appendix

2.5.1 Supplementary Methods

2.5.1.1 Statistical analysis using generalized linear mixed model (GLMM)

In Experiment 1 and 2, we focused on running distance and total time in which both chicks stayed in the same end of the maze (i.e. numerator of synchrony index) as response variables. In experiment 3, we focused on number of pecks, number of gained grains, running distance and velocity as response variables.

We assumed a poisson distribution for the error structure of the data of running distance, number of pecks, and number of gained grains, considering that they were all nonnegative values. $\Lambda(X)$ (>0) was thus approximated by a poisson function (log link function) as

$$\Lambda(X) = \exp(X) \qquad (1)$$

On the other hand, we assumed a binomial distribution for the error structure of the data of total time in which both chicks stayed in the same end of the maze, since the time was calculated by the number of video frames and all frames were fallen into either "same end" or "different end" category. Synchrony index = $Q(X)$ ($\in [0, 1]$) was thus approximated by a logistic function (logit link function) as

$$Q(X) = 1 / (1 + \exp(-X)) \qquad (2)$$

in which a predictor X was linearly given as a weighed sum of explanatory variables.

Experiment 1 : $\boldsymbol{X = \beta_0 + (\beta_1 + r_{is})*day + \beta_2*maze + \beta_3*cage} + r_{ii}$ (3)

day (variable = 1, 2, 3, 4, or 5) denotes experimental days. Coefficient β_1 indicates how the day contributes to running distance or synchrony index. A positive value of

estimated β_1 thus suggests that the running distance increases as the days elapses.

maze (categorical variable) denotes foraging condition in the maze (i.e. single or paired). Coefficient β_2 indicates how paired foraging in the maze contributes to the response variables. A negative value of estimated β_2 suggests that single chicks in the maze ran/synchronized less than paired chicks in the maze.

cage (categorical variable) denotes foraging condition in the cage when the main diet food was supplied (i.e. single or paired). Coefficient β_2 indicates how paired foraging in the cage contributes to the response variables. A negative value of estimated β_2 suggests that runs by single chicks in the cage ran/synchronized less than paired chicks in the cage.

Experiment 2 : $\boldsymbol{X = \gamma_0 + (\gamma_1 + r_{is}) * day + \gamma_2 * maze + \gamma_3 * feeder + r_{ii}}$ (4)

maze (categorical variable) denotes the type of wall in the maze (i.e. transparent or opaque). Coefficient γ_2 indicates how visual perception contributes to the response variables. A negative value of estimated γ_2 suggests that chicks mutually invisible ran/synchronized less than chicks mutually visible.

feeder (categorical variable) denotes whether the feeders were separated or shared. Coefficient γ_3 indicates how the actual competition over food intake contributes to the response variables. A negative value of estimated γ_3 suggests that the actual competition over food intake decreased running distance/synchrony less than chicks with no competition.

Experiment 3 : $\boldsymbol{X = \delta_0 + \delta_1 * maze + (\delta_2 + r_{is}) * feeder + \delta_3 * maze * feeder + r_{ii}}$ (5)

maze (categorical variable) denotes foraging condition in the maze (i.e. single or

paired).

feeder (numeric variable; 1, 2, 3, 4 and 5) denotes difficulty of food trays. Coefficient δ_2 indicates how increasing difficulty of food trays contributes to the response variables. A positive value of estimated δ_2 suggests that the more difficult the food tray was, the larger the response variable was.

Coefficient δ_3 indicates how *maze* and feeder interact.

The intercepts (β_0, γ_0 and δ_0) denote bias at the population level. The random intercept and the random slope (against day and feeder in Experiment 1, 2 and 3, respectively) for each individual (i) was denoted by r_{ii} and r_{is}, representing noise that was not experimentally controlled; Gaussian distribution with mean = 0 was assumed.

In experiment 1, AICs were compared among 8 models with different combination of parameters; (i) null model [β_0], (ii) day model [β_0, β_1], (iii) maze model [β_1, β_2], (iv) cage model [β_1, β_3], (v) day-maze model [β_0, β_1, β_2], (vi) day-cage model [β_0, β_1, β_3], (vii) maze-cage model [β_0, β_2, β_3] and (viii) day-maze-cage model [β_0, β_1, β_2, β_3].

Similarly in experiment 2, AICs were compared among the following 8 models; (i) null model [γ_0], (ii) day model [γ_0, γ_1], (iii) maze model [γ_0, γ_2], (iv) feeder model [γ_0, γ_3], (v) day-maze model [γ_0, γ_1, γ_2], (vi) day-feeder model [γ_0, γ_1, γ_3], (vii) maze-feeder model [γ_0, γ_1, γ_3], and (viii) day-maze-feeder model [γ_0, γ_1, γ_2, γ_3].

In experiment 3, AICs were compared among the following 5 models; (i) null model [δ_0], (ii) maze model [δ_0, δ_1], (iii) feeder model [δ_1, δ_2], (iv) maze-feeder model [δ_0, δ_1, δ_2], and (v) maze-feeder-and-interaction model [δ_0, δ_1, δ_2, δ_3].

Most likely values of the intercepts and coefficients (β_0, β_1, β_2, β_3, γ_0, γ_1, γ_2, γ_3 and

δ_0, δ_1, δ_2, δ_3) were estimated on the basis of the choice data by using R (version 2.12.0; R Development Core Team, 2010) and the lme4 package (version 0.999375-37; Bates & Sarkar 2010). AICs were given as a sum of the deviance plus 2 times the number of parameters. The AICs and the parameter estimates are shown in Table II-S1 to S2, S3 to S4, and S5 to S8 for experiment 1, 2 and 3, respectively. For interpretations of the statistic computations, see the main text.

In all tables, models are sorted in the order of AICs. Hyphen means that the model does not include the parameter. Coefficients in parentheses represents that the 95% confidence interval (CI) of the estimate included 0. CI was not considered for the intercepts (β_0, γ_0 and δ_0).

2.5.2 Referrences

Bates D. M. and Sarkar D. (2010) lme4: Linear mixed-effects models using S4 classes, R package version 0.999375-37. http://cran.r-project.org/web/packages/lme4/index.html

R Development Core Team (2010) R version 2.12.0.: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org.

2.6 Figures

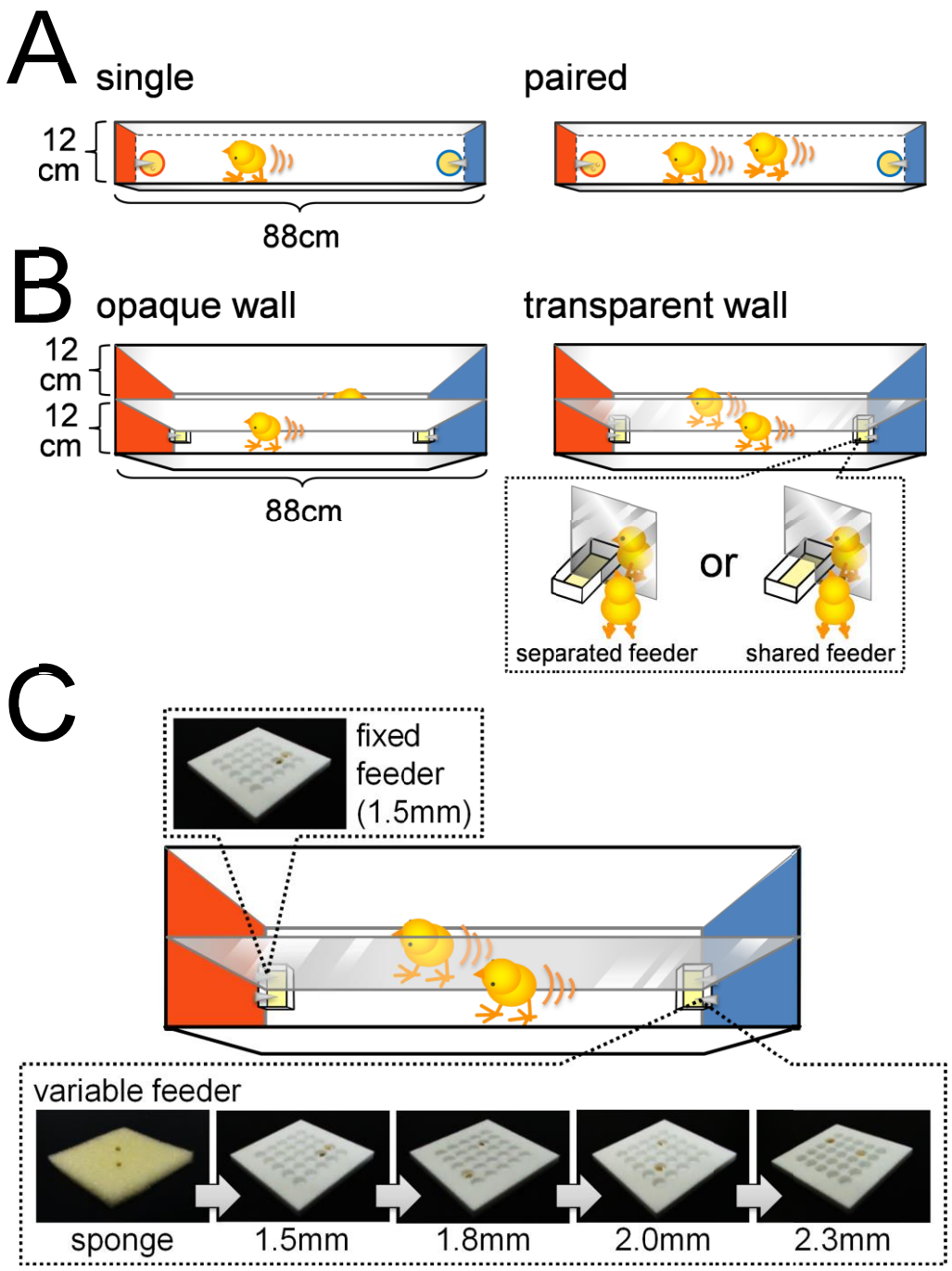


Figure II-1. Experimental apparatus for examining foraging efforts, running distance (A, B) and number of pecks (C).

(A) One-lane I-shaped maze equipped with a pair of terminal feeders. Terminal walls of

the maze were colored red and blue. The feeders supplied grains of millet according to a variable interval schedule. One grain was supplied at one time in the single-chick condition (left), and two grains were supplied at one time in the paired-chicks condition (right). Note that the paired chicks were competing over food, whereas the single chicks were not.

(B) Two-lane I-shaped maze. Chicks and feeders were separated by an opaque (left) or a transparent (right) wall. In the transparent wall condition, the feeders were either separated or shared. Note that the paired chicks in the shared feeder condition were competing for food, whereas chicks in the other condition did not.

(C) The 2-lane maze was equipped with a fixed feeder and a variable feeder. The food tray of the fixed feeder was made of a plastic plate with 25 holes (5x5), each 1.5 mm deep. The food tray of the variable feeder was either a sponge or plastic plates of variable depth (ranging from 1.5 to 2.3 mm). Supplied grains tumbled into the holes, making it difficult for chicks to obtain the grains. The deeper the holes were, the more difficult it was for chicks to obtain the grains. Trays of the variable feeder were sequentially replaced in an order from the easy sponge to the more difficult plates every ca. 3 min. See text for details.

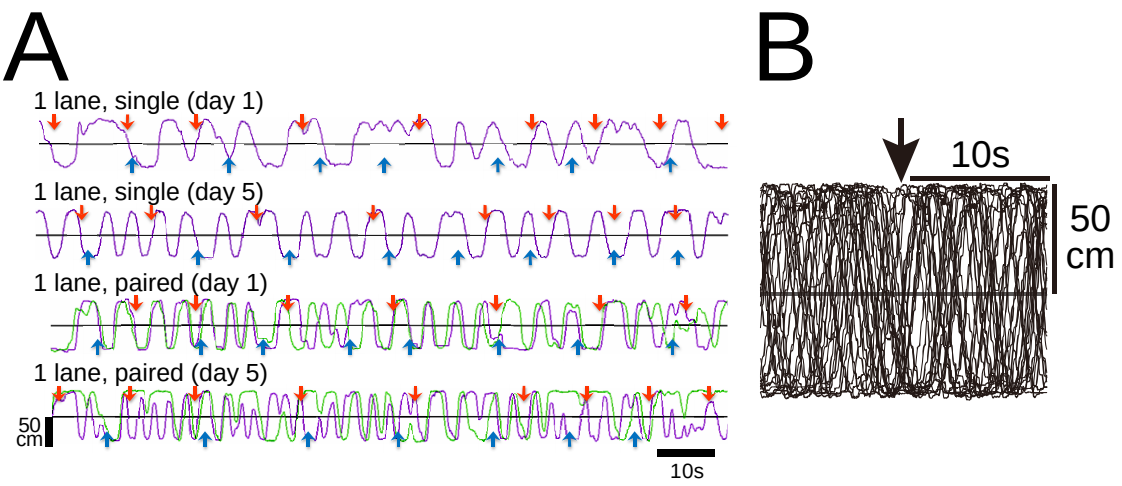


Figure II-2. Representative running trajectories in the 1-lane condition (Experiment 1).

(A) Two-min records of running trajectories. Purple and green lines represent the trajectory of individuals along the long axis of the maze; upward indicates the direction to the red feeder. Red and blue arrows indicate the time at which a grain(s) was delivered. The top and second records (1 lane, single) were obtained from the same chick on different days (days 1 and 5). The third and fourth records (1 lane, paired) were obtained from a pair of individuals. Comparison of the trajectories on day 1 (top vs. third) and day 5 (second vs. fourth) clearly shows that the paired chicks ran at a higher frequency.

(B) Thirty superimposed trajectories aligned at the time of grain delivery from the red feeder (downward arrow at the top). An example obtained from a chick tested in the single condition in the maze. Note that the runs were not in response to the food delivery.

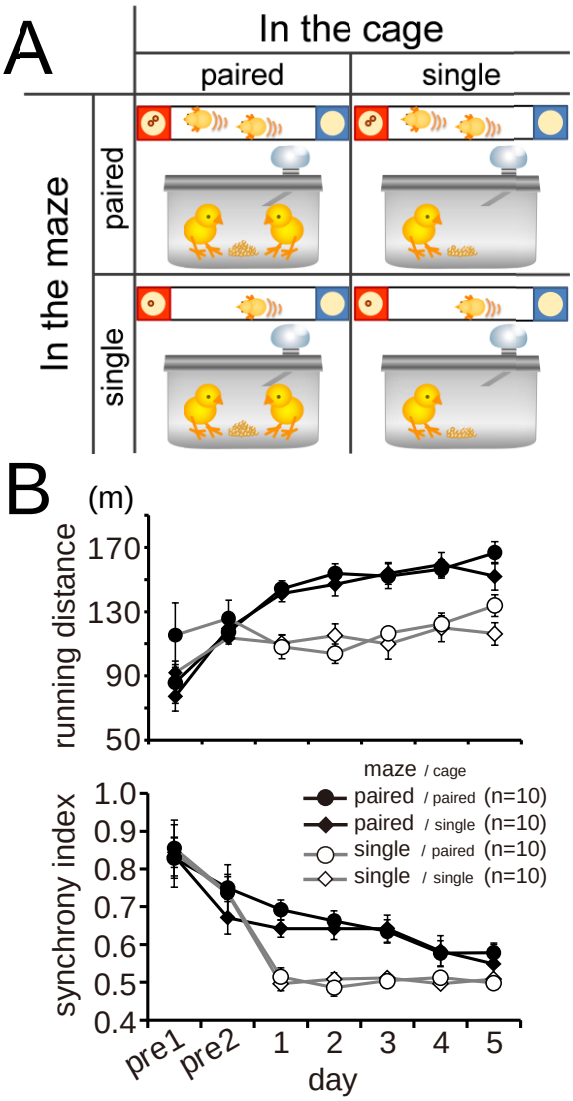


Figure II-3. Paired foraging in the maze, but not in the cage, influenced running (experiment 1).

(A) Chicks were divided into four experimental groups according to 2×2 block placement. "In the maze" means chicks were single or paired in the I-shaped maze during the tests, whereas "in the cage" means that chicks foraged in single or paired condition in their home cage. Note that chicks were housed in pairs in all conditions except foraging.

(B) Means (±SEM) of running distance (upper) and synchrony index (lower) during

feeding time (ca. 8 minutes) are plotted against the day of the experiment. Open and filled symbols denote paired and single foraging in the maze, whereas circles and rhombi denote paired and single foraging in the cage, respectively, in this and the following figures.

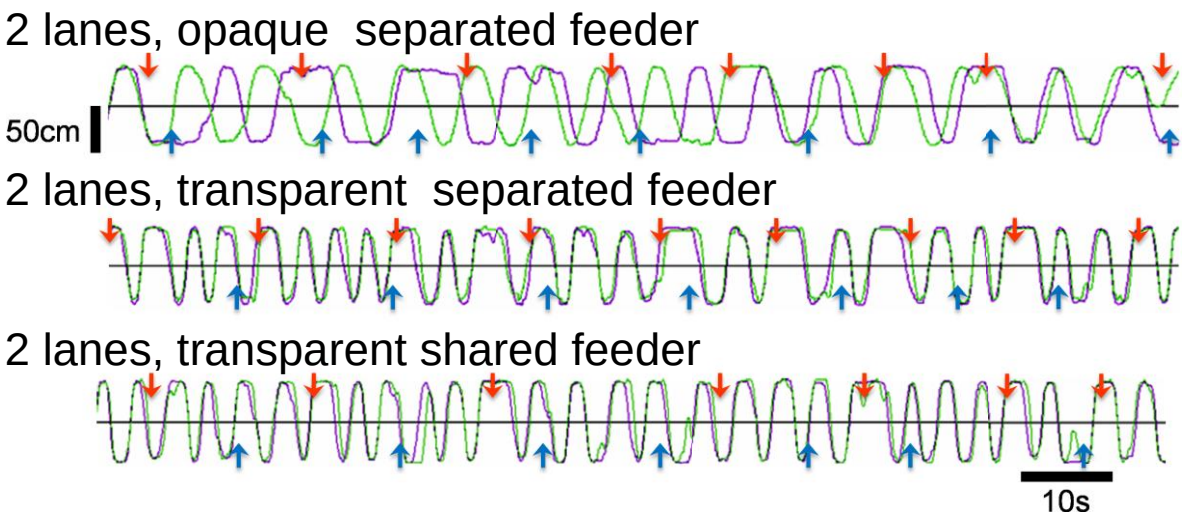


Figure II-4. Representative running trajectories in the 2-lane condition (Experiment 2).

When separated by an opaque wall, running trajectories were not synchronized (top). When separated by a transparent wall, runs were more frequent and synchronized regardless of whether the feeder was separated (middle) or shared (bottom). All records were obtained on experimental day 5.

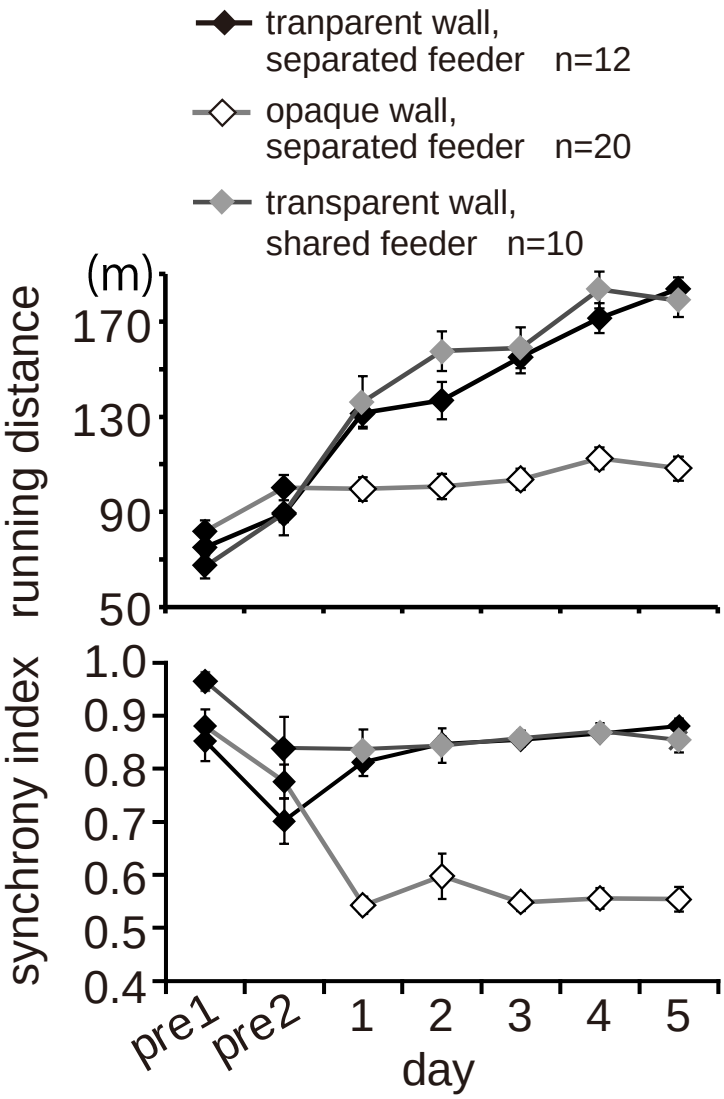


Figure II-5. Social interactions via the transparent wall caused a high degree of synchrony regardless of foraging competition (Experiment 2).

Means (±SEM) of running distance (upper) and synchrony index (lower) are plotted against the day of the experiment. Open and filled symbols denote data in the opaque wall and the transparent wall, respectively. Gray symbols denote the data obtained from another group in which the feeders were shared and the wall was transparent.

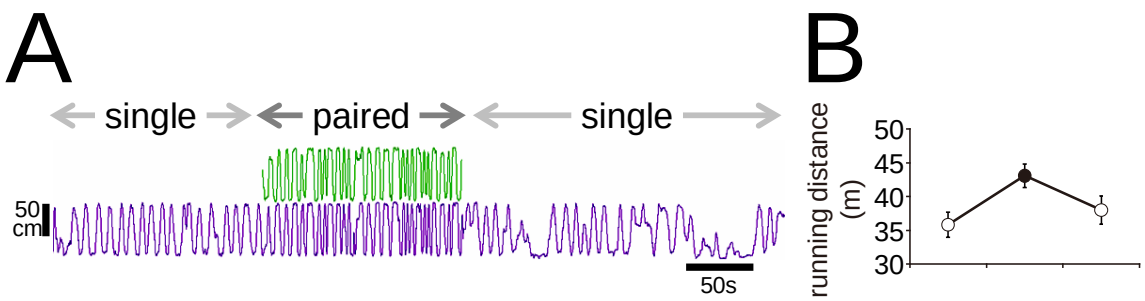


Figure II-6. The run was facilitated as soon as a companion chick was introduced (Experiments 1 and 2).

(A) Representative running trajectories of a pair of chicks. Subject chick (purple) received 30 deliveries of food, divided into three phases. In the first phase, the subject was tested in single condition. In the second phase, a companion chick (green) was introduced into the maze. In the third phase, the subject was again tested in single condition. Each phase lasted for ca. 3 min.

(B) Means (±SEM) of running distance recorded in the first and third phases of the single condition (open symbols) and in the second phase of the paired condition (filled symbol).

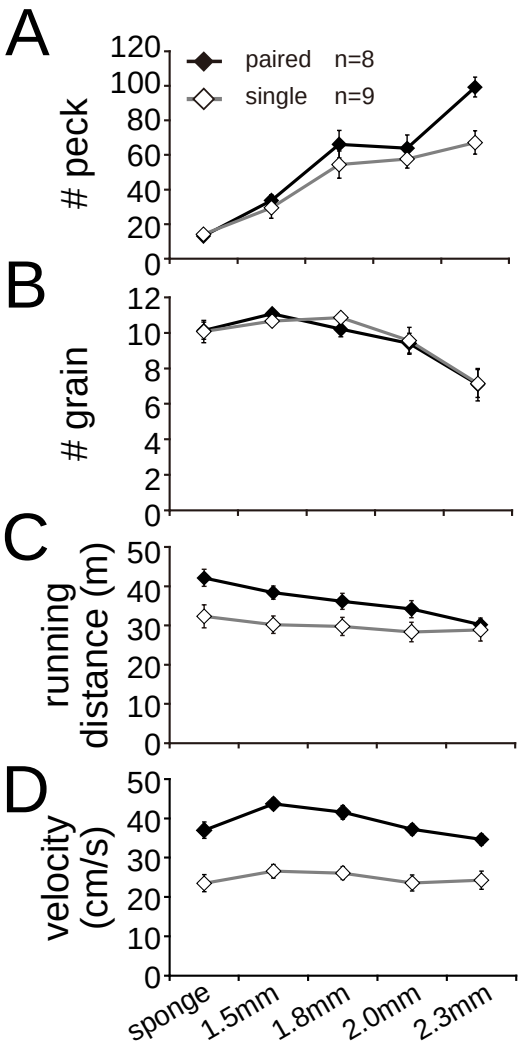


Figure II-7. Social interactions of pairing were also found in the number of pecks (handling effort), particularly in the difficult food condition (Experiment 3). Means (±SEM) of two groups of chicks (paired (filled symbols) and single conditions (open symbols)) are plotted against the phases of different food tray difficulty (from the sponge to the plate with deep holes). Each individual chick was repeatedly tested on 3 successive days, and the values were averaged to yield individual data. Number of pecks (A), total gain of grains (B), running distance (C), and mean velocity (D) are plotted. Note that the chicks spent a shorter time for running but ran at higher velocity in the paired condition, even in the difficult condition.

2.7 Tables

Table II-S1. The *day-maze* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 8 models designed for running distance in Experiment 1. The models are sorted in ascending order of AIC. Coefficients in parentheses represents that the 95% confidence interval (CI) of the estimate included 0. The model [β_0, β_1, β_2] indicates that both *day* and *maze* had significant effects, whereas the same-AIC. Model [β_0, β_1, β_2, β_3] indicates that *cage* was not reliable for its coefficient.

Most-likely-fitting formulas are indicated below the table.

models		AIC	estimated coefficients of variables			
			β_0 [*intercept*]	β_1 [*day*]	β_2 [*maze*]	β_3 [*cage*]
1	[β_0, β_1, β_2]	14794	9.475460	0.033304	-0.347460	-
2	[β_0, β_1, β_2, β_3]	14794	9.442871	0.033308	-0.347421	(0.065203)
3	[β_0, β_2]	14812	9.47573	-	-0.34738	-
4	[β_0, β_2, β_3]	14813	9.44303	-	-0.34736	(0.06539)
5	[β_0, β_1]	14822	9.301773	0.033290	-	-
6	[β_0, β_1, β_3]	14823	9.269186	0.033299	-	(0.065212)
7	[β_0]	14841	9.30201	-	-	-
8	[β_0, β_3]	14842	9.26935	-	-	(0.06536)

Table II-S2. The *day-maze* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 8 models designed for synchrony index (proportion of the number of video flames in which chicks were in the same end of the maze) in Experiment 1. The model [β_0, β_1, β_2] indicates that both *day* and *maze* had significant effects, whereas the second-smallest-AIC model [β_0, β_1, β_2, β_3] indicates that *cage* was not reliable for its coefficient.

models		AIC	estimated coefficients of variables			
			β_0 [*intercept*]	β_1 [*day*]	β_2 [*maze*]	β_3 [*cage*]
1	[β_0, β_1, β_2]	4834	0.85135	-0.05698	-0.83931	-
2	[β_0, β_1, β_2, β_3]	4835	0.89544	-0.05698	-0.83924	(-0.08825)
3	[β_0, β_2]	4839	0.85106	-	-0.83970	-
4	[β_0, β_2, β_3]	4840	0.89519	-	-0.83963	(-0.08833)
5	[β_0, β_1]	4862	0.43171	-0.05700	-	-
6	[β_0, β_1, β_3]	4864	0.47585	-0.05698	-	(-0.08837)
7	[β_0]	4867	0.4311	-	-	-
8	[β_0, β_3]	4869	0.47541	-	-	(-0.08846)

Table II-S3. The *day-maze* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 8 models designed for running distance in Experiment 2. The model [γ_0, γ_1, γ_2] indicates that both *day* and *maze* had significant effects, whereas the second-smallest-AIC model [γ_0, γ_1, γ_2, γ_3] indicates that *feeder* was not reliable for its coefficient.

models		AIC	estimated coefficients of variables			
			γ_0 [*intercept*]	γ_1 [*day*]	γ_2 [*maze*]	γ_3 [*feeder*]
1	[γ_0, γ_1, γ_2]	16191	9.320326	0.060972	-0.276690	-
2	[γ_0, γ_1, γ_2, γ_3]	16192	9.270098	0.060971	-0.226365	(0.110372)
3	[γ_0, γ_1, γ_3]	16196	9.128636	0.060968	-	0.251822
4	[γ_0, γ_1]	16201	9.188624	0.060951	-	-
5	[γ_0, γ_2]	16228	9.32036	-	-0.27610	-
6	[γ_0, γ_2, γ_3]	16229	9.27008	-	-0.22573	(0.11048)
7	[γ_0, γ_3]	16233	9.12902	-	-	0.25154
8	[γ_0]	16237	9.18896	-	-	-

Table II-S4. The *day-maze* model yielded the smallest AIC, but the effect of *day* was not reliable for its coefficient.

AICs and estimated coefficients of variables were calculated for 8 models designed for synchrony index in Experiment 2. The model [γ_0, γ_1, γ_2] indicates that *day* had insignificant effects.

models	AIC	estimated coefficients of variables			
		γ_0 [*intercept*]	γ_1 [*day*]	γ_2 [*maze*]	γ_3 [*feeder*]
1 [γ_0, γ_1, γ_2]	14088	1.54956	(0.04025)	-1.29326	-
2 [γ_0, γ_2]	14089	1.5501	-	-1.2934	-
3 [γ_0, γ_1, γ_2, γ_3]	14089	1.42322	(0.04025)	-1.16667	(0.27757)
4 [γ_0, γ_2, γ_3]	14090	1.4236	-	-1.1667	(0.2782)
5 [γ_0, γ_3]	14105	0.6943	-	-	1.0073
6 [γ_0, γ_1, γ_3]	14105	0.69392	(0.04026)	-	1.00706
7 [γ_0, γ_1]	14110	0.93383	(0.04022)	-	-
8 [γ_0]	14111	0.9340	-	-	-

Table II-S5. The *maze-feeder-and-interaction* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 5 models designed for number of pecks in Experiment 3. The model [δ_0, δ_1, δ_2, δ_3] indicates that *maze* per se had no effects in the absence of feeder.

models		AIC	estimated coefficients of variables			
			δ_0 [*intercept*]	δ_1 [*maze*]	δ_2 [*feeder*]	δ_3 [*maze:feeder*]
1	[δ_0, δ_1, δ_2, δ_3]	434.8	2.67886	(0.04795)	0.39100	-0.07668
2	[δ_0, δ_2]	437.7	2.69015	-	0.35387	-
3	[δ_0, δ_1, δ_2]	439.4	2.74001	(-0.09332)	0.35381	-
4	[δ_0]	490.3	2.6991	-	-	-
5	[δ_0, δ_1]	492.3	2.67413	(0.04772)	-	-

Table II-S6. The *feeder* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 5 models designed for total gain of grains in Experiment 3. The model [δ_0, δ_2] indicates that only *feeder* had significant effects.

models	AIC	estimated coefficients of variables			
		δ_0 [*intercept*]	δ_1 [*maze*]	δ_2 [*feeder*]	δ_3 [*maze:feeder*]
1 [δ_0, δ_2]	42.02	2.48704	-	-0.07750	
2 [δ_0, δ_1, δ_2]	44.01	2.483046	(0.007534)	-0.077502	
3 [δ_0, δ_1, δ_2, δ_3]	45.98	2.494080	(-0.013251)	-0.081384	(0.007306)
4 [δ_0]	49.77	2.26054	-	-	
5 [δ_0, δ_1]	51.76	2.25654	(0.00753)	-	

Table II-S7. The *maze-feeder-interaction* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 5 models designed for running distance in Experiment 3. The model [δ_0, δ_1, δ_2, δ_3] indicates that *maze*, *feeder* and their interaction had significant effects.

models		AIC	estimated coefficients of variables			
			δ_0 [*intercept*]	δ_1 [*maze*]	δ_2 [*feeder*]	δ_3 [*maze:feeder*]
1	[δ_0, δ_1, δ_2, δ_3]	1640	8.41344	-0.34862	-0.07784	0.04685
2	[δ_0, δ_1, δ_2]	1643	8.41253	-0.34666	-0.05309	-
3	[δ_0, δ_2]	1650	8.22898	-	-0.05308	-
4	[δ_0, δ_1]	1655	8.41218	-0.34806	-	-
5	[δ_0]	1662	8.22792	-	-	-

Table II-S8. The *maze* model yielded the smallest AIC.

AICs and estimated coefficients of variables were calculated for 5 models designed for running velocity in Experiment 3. The model [δ_0, δ_1] indicates that only *maze* had significant effects.

models		AIC	estimated coefficients of variables			
			δ_0 [*intercept*]	δ_1 [*maze*]	δ_2 [*feeder*]	δ_3 [*maze:feeder*]
1	[δ_0, δ_1]	77.45	3.65249	-0.45497	-	-
2	[δ_0, δ_1, δ_2]	77.58	3.70838	-0.45496	(-0.01875)	-
3	[δ_0, δ_1, δ_2, δ_3]	78.84	3.73782	-0.52555	(-0.02872)	(0.02379)
4	[δ_0]	93.85	3.41092	-	-	-
5	[δ_0, δ_2]	93.98	3.46683	-	(-0.01875)	-

CHAPTER III

Distinct roles of medial and lateral dopaminergic pathways on social facilitation

3.1 Introduction

Despite extensive studies on behavioral and cognitive bases of social facilitation (Zajonc 1965, Strauss 2002 for review), its neural substrates remain still elusive. In a previous study (Ogura & Matsushima 2011, Chapter 2), we found that chicks paid foraging effort only during food delivery, thus its social facilitation could also be controlled by way of the reward-based behavioral execution system in the brain. However, we also found that the social facilitation occurred even without an increase in food gain. The social facilitation may thus occur independently of the reward system, and separate neural system might have to be considered.

One of the key structures of the reward system is the medial striatum (MSt; Figure III-1A), that is homologous to the ventral striatum including nucleus accumbens in mammals. Single unit recording experiments revealed that the chick MSt is involved in evaluation and anticipation of reward (food and water; Yanagihara et al. 2001, Izawa et al. 2005). Similarly to the ventral striatum in mammals (Haber et al. 2000), the avian MSt receives dense dopaminergic projections from midbrain ventral tegmental area (VTA) (Figure III-1A, B; Durstewitz et al. 1999, Mezey & Csillag 2002). The VTA-MSt dopaminergic pathway is also assumed to be involved in the reward prediction in primates (Schultz 1998, 2007). Furthermore, dopamine transmission in the ventral striatum is critical for adaptive control of effort for food in rat (Salamone et al. 1991, 1994, 2007).

Another major dopaminergic pathway should also be considered, and it is the pathway from substantia nigra (SN) to the lateral striatum (LSt; homologous to dorsal striatum in mammals; Figure III-1A, B). The lateral SN-LSt pathway is anatomically

distinct from the medial VTA-MSt pathway, though a considerable overlaps occur between them (Figure III-1A, Mezey & Csillag 2002, Bálint et al. 2004). In mammals including humans, similarly, anatomical (Haber et al. 2000) and functional (O'Doherty et al. 2004) dissociation has been demonstrated between the medial (to the ventral striatum) and the lateral dopamine pathways (to the dorsal striatum). In birds, on the other hand, little is known about functional dissociation of these two pathways.

As a step to identify the neural substrate of social facilitation, we made localized lesion in medial and lateral pathways, and investigated the effect on the foraging effort and its social facilitation in chicks. For the medial pathway, electrolytic lesion and dopamine-selective lesion were made in MSt by using neurotoxic 6-hydroxydopamine (6-OHDA). For the lateral pathway, electrolytic lesion and 6-OHDA lesion were made in SN.

3.2 Materials and Methods

3.2.1 Subjects

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, chicks were sacrificed in carbon dioxide according to the guidelines.

Male domestic chicks (*Gallus domesticus*, White Leghorn strains) were used. New hatchlings of post-hatch day 1 (presumed hatching day) were obtained from a local supplier (Iwamura poultry Ltd. Hokkaido establishment, Yubari, Hokkaido, Japan). Chicks were paired and housed in transparent plastic cages (15 cm × 28 cm × 12 cm) under illumination using white LED lamp (12L: 12D; light period starting at 08:00) and thermo-controlled at *ca.* 28°C. Water was available *ad libitum*. Two types of food were given, i.e., grains of millet and chick mash food. The total amount of food per day was kept at a certain level so that (1) the body weight of chicks gradually increased and (2) the chicks actively consumed food during experiments. From post-hatch day 1, chicks were fed mash food. The amounts of mash food were 2 g (post-hatch days 1-3) and 2.5 g (from day 4). From post-hatch day 2, grains of millet were added. The amounts of grains (per chick per day) were 2 g (days 2 and 3) and 2.5 g (from day 4). Until day 2, all chicks were communally fed. From day 3, chicks were solitarily fed. Chicks were fed in a cage that was visually separated by a black plastic wall, so that chicks did not see the other chicks eating food. Chicks were communally housed except when the daily diet was given.

3.2.2 Apparatus

An I-shaped maze equipped with parallel two lanes (Figure III-2A; 12 cm in width per lane, 88 cm in length, and 40 cm in height) was used. These two lanes were separated by a transparent acryl board. Walls at the terminals were colored in red (left) or blue (right). The maze was equipped with a pair of terminal feeders for each lane. Each feeder supplied a grain of millet food at a time, and the interval was set variable. If not stated otherwise, the interval was uniformly distributed from 10 to 20 sec (mean = 15 sec), and it was referred to as VI15. Two food trays were placed in adjacent positions over the separation board. Rectangle food trays (3 cm in width, 4 cm in length, and 2 cm in depth) with sponge on the floor were used. In order to prevent chicks to associate the feeder sound with food reward, sounds of electric motors (placed around the maze) were replayed at variable intervals(uniformly distributed from 1.5 to 3.5 sec with the mean = 2.5 sec). The apparatus was placed in a dark room kept at ca. 25–30°C and illuminated under four 60-W white light bulbs placed above the runway and feeders. Timing of grain delivery and the noise sound were controlled by microrobots (RCX, LEGO Mindstorms).

Behavior of the chicks was recorded using a video handy cam (DCR-SR65, Sony, Japan) at 30 frames per sec for offline analysis. The handy cam was located just above the I-maze, providing an aerial view of the subject and companion chicks. Chick was individually marked by a rectangular small piece of fluorescent-colored tape (Yamato Co., Ltd., Japan) affixed on its head. The position of the fluorescent markers was analyzed by using Move-Tr/2D 7.0 software (Library Co., Japan) and the trajectories and running distances were thus calculated.

3.2.3 Behavioral procedures

3.2.3.1 Habituation

Naïve chicks (post-hatch days 4-7) were initially habituated to the I-maze for two successive days before tests (pre-1 and pre-2). Paired chicks (housed in the same cage) were placed on the midway of the maze, in which some food (ca. 100 grains of millet) was given. After the chicks had consumed the food, feeders started to deliver food according to the VI15 schedule (mean interval of 15 s, uniformly distributed in a range of 10–20 s). Two grains were supplied to the pair in each delivery, so that each chick was expected to gain 1 grain.

3.2.3.2 Test

3.2.3.2.1 Effects of paired foraging on running distance

Subjects received tests for 8 times; 4 tests in pre-operative (i.e., pre-surgery) days and another set of 4 tests in post-operative session. Running distance was measured as an index of effort paid for approaching food. On the next day after the habituation, behavioral tests were conducted for 2 consecutive days, 2 test per day. Surgical operation was conducted on the day after the second pre-operative test. Two days after the surgery, post-operative session was conducted, in which subjects were tested in 4 tests in 2 consecutive days. Each test was composed of 3 phases, namely, 1st-single, paired, and 2nd-single (Figure III-2A, top, middle, and bottom, respectively). Initially, subject was tested singly in the maze. To acclimatize the chick, *ca.* 20 grains/chick placed in each feeder in advance, and the subject consumed all of the food available. Afterwards, subsequent 1 min of short habituation period, each of the two feeders

supplied 1 grain at a time in VI15 schedule. The 1st-single phase consisted of 30 times of programmed food delivery, and it lasted for *ca.* 8 minutes.). The companion chick (cagemate) was thereafter placed in another lane of the maze, and a paired phase started. During the paired phase, 30 grains were delivered from each feeder for both subject chick and the companion chick. Finally the companion was removed from the maze and the subject singly foraged again for 30 times (2nd-single phase). After the feeding was over, subject chick was left in the maze for an additional 2 min after it consumed all grains delivered on the food trays, and the test session was terminated. As each phase took for *ca.* 8 min, it took *ca*. 26 minutes for one test.

3.2.3.2.2. Effects of feeding rate on running distance

After the final test was over, sham-operated chicks underwent a single foraging test that was consisting of 3 phases with different feeding rate. In the first and third phase, feeding frequency was set relatively low at VI30 for 15 times. In the second phase, a higher feeding rate was set at VI10 for 45 times. Each of the three phases lasted for *ca.* 8 minutes. The test was terminated at 2 min after subject consumed all of the delivered food.

3.2.4 Surgery

Chicks were anesthetized by intramuscular injection of *ca.* 0.4ml of ketamine-xylazine cocktail (a 1:1 mixture of 10 mg/ml ketamine [Daiichi Sankyo Co. Ltd.] and 2 mg/ml xylazine [Sigma-Aldrich Co. LLC, St. Louis, MO]). Supplementary injections (0.10 ml) were given to maintain a stable anesthesia. Chicks were then fixed on a stereotaxic apparatus. Skin over the skull surface was incised, and the dura mater was

cut to expose the brain.

For electrolytic lesion, a sharp steel electrode made from insect pin (size #00; Shiga Konchu, Tokyo, Japan) coated by enamel paint (Tamiya, Inc., Shizuoka, Japan) except for 0.5 mm at the tip. For MSt lesion, electrode was inserted at the following coordinates: anterior-posterior (ap) +3.3 to +3.7mm from bregma, 1.0mm lateral from midline, 6.2 to 6.5 mm deep from brain surface. For SN lesion, the coordinates were; ap +0.8 to -0.3 mm from bregma, 1.75 to 2.0mm lateral from midline, 8.0 to 8.8 mm from brain surface.

To selectively deplete dopaminergic neurons and terminals, 6-hydroxydopamine hydrobromide (6-OHDA; Sigma-Aldrich) was infused to MSt or SNc by using a 1μl-Hamilton micro-syringe and an motor-driven injector. For single injection site, 0.6μl of 6-OHDA solution was infused for a period over 13 min, so that a total of 6μg (MSt) or 12μg (SN) was infused per site. As the vehicle, 0.2% ascorbic acid in saline (0.9% NaCl) was used. After the end of infusion, syringe was left in the brain for an additional 5 min for diffusion of the infused drug. For MSt lesion, following coordinates were adopted: ap +3.3 to +3.4 mm from bregma, 1.0 mm lateral from midline, 5.9 to 6.2 mm deep from brain surface. For SN lesion, the coordinates were: ap +0.3 to -0.3 mm from bregma, 2.0 mm lateral from midline, 8.2 to 8.5 mm deep from brain surface.

For sham operation, a total of 11 chicks received either insertion of an electrode without current or injection of 0.6μl ascorbic acid solution (vehicle) into either MSt or SN.

The incised skull was covered with gelatin sponge (Spongel®, Astellas, Japan), and the skin flap was fixed using superglue (Aron Alpha®, Toa-Gosei, Japan). The operated-on chicks were then housed in the breeder for recovery. The anesthesia lasted

for 5 to 6 hours. After the recovery, and chicks showed normal posture and locomotor activity, and actively foraged food without showing any motor deficiency.

3.2.5 Histology

Two days after the final test (i.e., 4th test of the post-operative session), chicks were deeply anesthetized by 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 lesion, brain tissue (telencephalon and midbrain) was embedded in yolk and post-fixed in the same fixative for 2 or more days at 4°C. The tissue was cut into 100μm frontal sections by a vibrating microslicer (DTK-1000, Dosaka EM, Japan). Sections were mounted and stained with cresyl violet.

In chicks with 6-OHDA lesion and electrolytic lesion of SN, brain tissue was examined also by tyrosine hydroxylase (TH) immunohistochemistry. Chicks were perfused and the dissected brain tissue was post-fixed for 6 hours at 4ºC, and then in 20% sucrose in PBS at 4 ºC for overnight. Brain tissue was frozen at -80 ºC for storage, and cut into 40 μm frontal sections by a freezing microtome (Yamato Kohki Industrial Co. Ltd., Japan). Floating sections were stained with antibody (anti-TH rabbit polyclonal antibody, diluted x1,000, Merck Millipore Co. Billerica, MA; former Chemicon, Temecula, CA) and with secondary antibody (biotinylated goat anti-rabbit IgG; diluted x300, ABC kit, Vector Laboratories, Inc., Burlingame, CA). Reaction product was visualized with DAB, and the sections were mounted, dehydrated, and cleared. Terminology of neural nuclei and definition of their boundaries followed the stereotaxic atlas of chick brain by Kuenzel and Masson (1988).

3.3 Results

A total of 100 male chicks were used. Of these, 46 chicks served as experimental subjects and another 46 served as companions. The remaining 8 chicks were discarded, as they died after surgery (3 chicks), or they showed a general motor deficiencies (2 chicks). In addition, 3 companion chicks died before the end of experiment. All companions were the cage-mate of the subjects and fixed throughout the experiment.

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

In accordance with my previous report (Ogura & Matsushima 2011, chapter 2), the sham subjects (n=11) instantaneously increased their running distance when a companion chick was introduced, and they decreased it when the companion was removed. Figure III-2B shows an example, and Figure III-2D shows population data obtained from 11 sham-operated chicks. Data obtained in 4 post-operative tests were averaged in each individual, and data obtained from these 11 chicks were superimposed. Friedman test revealed a significant difference among three test phases (χ^2=18.7273, *df*=2, *p*=0.0001). Post-hoc pairwise comparisons using Wilcoxon signed rank test with Holm correction revealed significant differences between the first single phase (single 1) and the paired phase (**, *p*=0.0029) and between the second single phase (single 2) and the paired phase (**, *p*=0.0029), but no significant difference was found between these single phases (n.s., *p*>0.05). Social facilitation of running distance was thus reconfirmed, and effect of test repetition was not found.

Instantaneous increase in running distance was found also when feeding rate was increased in the single chick condition. After the social facilitation tests were completed,

9 out of the 11 sham-operated chicks were tested in a continuous series of VI30, VI10, and VI30 phases. Figure III-2C shows an example, and population data are shown in Figure III-2E. Friedman test revealed a significant difference among the three test phases (χ^2=13.5556, *df* = 2, *p*=0.0011) and post-hoc pairwise comparisons using Wilcoxon signed rank test with Holm correction revealed significant differences between the VI10 phase and each of the two VI30 phases (*, *p*=0.0120), but no significant difference was found between the two VI30 phases (n.s., *p*>0.05). Although smaller in magnitude, a rise in feeding rate caused a similar increase in running distance, and the effect of test repetition was not found.

We therefore assumed the following two variables as independent behavioral indices, namely (1) single running distance as an index of effort for food reward, and (2) increase in running distance in paired phase as an index of social facilitation. The socially facilitated increase (Δ of running distance) was given as;

$$\Delta\ of\ running\ distance = paired - \frac{single\ 1 + single\ 2}{2}$$

Actually, no significant correlation was found between these indices in the sham-operated chicks (Figure III-2F; *t*=0.1903, *p*>0.05, n=11).

3.3.2 Effects of MSt and SN lesions on running distance

Single running distance was compared among sham control group (n=11) and four groups of lesion, namely, MSt-electrolytic lesion (n=8), MSt-6-OHDA lesion (n=8), SN-electrolytic lesion (n=13) and SN-6-OHDA lesion (n=6). In the pre-operative session (Figure III-3A), Kruskal-Wallis test revealed no significant difference among the 5 groups (χ^2=9.4437, *df*=4, *p*>0.05). In the post-operative session (Figure III-3C), on the other hand, chicks with the MSt-electrolytic lesion showed significantly lower

single running distance than the sham control. Kruskal-Wallis test revealed a significant difference among the 5 groups of chicks (χ^2=22.4599, *df*=4, *p*=0.0002). Subsequent Steel's multiple comparison test indicated significantly lower single running distance in the MSt-electrolytic lesion than the sham control (**, *p*=0.0015), whereas no significant difference was detected in any other lesion groups.

We also tried bilateral electrolytic lesion of SN, but it resulted in aphagia (*i.e.* no spontaneous pecking for food or water) and severe debilitation in 3 out of 5 operated chicks. Measuring foraging effort from these subjects was impossible.

3.3.3 Effects of MSt and SN lesions on social facilitation

Δ of running distance was also compared among sham and four lesion groups. In the pre-operative session (Figure III-3B), Kruskal-Wallis test revealed no significant difference between the groups (χ^2=5.3877, *df*=4, *p*>0.05). In the post-operative session (Figure III-3D), on the other hand, both of the SN-electrolytic lesion and SN-6-OHDA lesion groups showed significantly lower Δthan the sham control. Kruskal-Wallis test (χ^2=20.1476, *df*=4, *p*=0.0005) and subsequent Steel's multiple comparison test revealed significantly lower Δ in both groups of SN- lesion (electrolytic lesion: **, *p*=0.0011; 6-OHDA lesion: **, *p*=0.0097), whereas no significant difference was detected in both groups of MSt-lesion.

3.3.4 Histological examination of lesions

Inter-individual behavioral variance could be ascribed to difference in lesion areas. We therefore superimposed the lesion areas separately for those individuals with large and small/none behavioral effects. Based on the mean and the unbiased variance

of sham control, we estimated the range in which 95% of intact subjects fall. For the single running distance, the range was 87.8-143.7. Similarly, for the Δ of running distance, the range was 56.6-98.7. In the group of MSt-electrolytic lesion, 7 chicks (out of n=8) fell below the estimated range of single running distance, thus were classified as those with a large effect (Figure III-4b). In the groups of SN-electrolytic lesion and SN-6-OHDA lesion, similarly, 10 chicks (out of 13) and 4 chicks (out of 6) fell below the estimated range of Δ of running distance, thus were classified as those with a large effect (Figure III-5b, d).

In the group of MSt-electrolytic lesion (Figure III-4b), dorsal part of MSt was completely destroyed in the large effect chicks, while it was intact in the remaining one chick (Figure III-4c). On the other hand, complete dopamine depletion was observed in both of the dorsal and ventral MSt in the MSt-6-OHDA lesion (Figure III-4d). Note that mechanical lesion was not detected in Nissl-stained sections.

In the group of SN-electrolytic lesion, dorsolateral SNc/SNr (Figure III-5b; A4.0 and A3.4) and posterior SNc (Figure III-5b, A2.8) were destroyed in the large effect chicks, whereas these areas were almost intact in the small/none effect chicks (Figure III-5c). In the group of SN-6-OHDA lesion, mechanical lesion around the posterior SNc was observed in all subjects (Figure III-5d and 5e). The lesion areas were located slightly more dorsal in the large effect chicks compared to the small/none effect chicks.

3.4 Discussion

The present results demonstrate a functional separation of the neural substrate of social facilitation from that of the reward-based foraging effort. Electrolytic lesion of MSt suppressed the single running distance that is supposed to be a behavioral index of the reward-based foraging effort. On the other hand, SN lesions did not cause a similar suppression (Figure III-3C). The 6-OHDA lesion of MSt did not change the single running distance, so that foraging effort may be independent of dopaminergic projection to MSt. On the other hand, both groups of chicks with SN lesions (electrolytic and 6-OHDA lesions) showed a suppression in the social facilitation, while the single running distance remained unchanged. The MSt lesions did not suppressed the social facilitation (Figure III-3D).

However, concerns still remain on the functional dissociation. First of all, effects of selective and localized VTA lesion should be examined to reconfirm the conclusion. Dopaminergic VTA neurons mainly project to MSt, whereas dopaminergic neurons in SN project to LSt (Mezey & Csillag 2002, Bálint et al. 2004). If the medial and the lateral pathways were functionally dissociated, VTA lesion should cause no effect on social facilitation. Actually, our preliminary results revealed no effects on social facilitation following VTA lesion.

Second, since 6-OHDA lesion in SN gave rise to some mechanical damage, the suppressed social facilitation might not be due to dopamine depletion, but to damages of other non-dopaminergic neurons or passing fibers in/around SN. In accordance with this idea, SN 6-OHDA lesion caused milder behavioral effects than SN electrolytic lesion in this study. To verify this point, we should deplete dopaminergic neurons in SN without

other general damages. It is also to be noted that the lesion extended wide to both substantia nigra pars compacta (SNc) and reticulata (SNr). Although these two nuclei are contiguous (Reiner et al. 2004), they have different anatomical projection patterns, thus considered to have different functions. Actually, majority of the SNr neurons are known to be GABAergic (Veenman & Reiner 1994, Sun et al. 2005) and density of dopaminergic cell bodies is lower when compared to those in SNc (Figure III-5a, A2.8-A3.4). We will discuss this issue again below.

Third, we have not accomplished an experiment in which dopaminergic projection to LSt was selectively lesioned. We are thus unable to conclude the role of dopaminergic modulation in LSt on social facilitation. Although SN sends massive dopaminergic projections to LSt, other telencephalic regions including arcopallium (Mezey & Csillag 2002) and nucleus accumbens shell (Bálint et al. 2011) also receives dopaminergic input from SN. In particular, dopaminergic projection to arcopallium could contribute to the facilitation of actions, since arcopallium has motor output toward brain stem and rostral spinal cord (Zeier & Karten et al. 1971).

Fourth, we should admit the ambiguity of our conclusion on the SN involvement of social facilitation, because only the right SN was unilaterally lesioned in this study. We were unable to examine behavioral effect of bilateral SN lesion, because preliminary study revealed a severe aphagia and debilitation. Considering no anatomical asymmetry found in the midbrain dopaminergic neurons, unilateral lesion for left SN may be supposed to induce a similar suppression of social facilitation. However, as functional lateralization is widely reported in the avian brain (Deng & Rogers 2002, Manns & Güntürkün 2003, Andrew et al. 2004), effects of the electrolytic and/or 6-OHDA lesion of the left SN should be examined.

Finally, damage to the noradrenaline pathway, rather than the dopaminergic, might be involved in the suppressed social facilitation. Since 6-OHDA is a general neurotoxin for catecholaminergic neurons, noradrenergic neurons should also be damaged in this study (Jonsson 1980). In this study we did not administer antidepressants to protect noradrenergic neurons, as reported in earlier studies (Walton et al. 2005, 2009). Actually, even under the treatment of antidepressants, 6-OHDA induces significant reduction of noradrenaline as well as dopamine (Walton et al. 2005, 2009). Thus, we admit that precise separation of dopaminergic and noradrenalinergic pathways is difficult in experiments using 6-OHDA as neurotoxin. However, although noradrenergic fibers and terminals project to SNc, dopaminergic neurons outnumber the noradrenergic cell bodies in the whole brain of chicken (Moons et al. 1995). Thus, behavioral effect of noradrenaline depletion, if any, might be negligible.

3.4.1 Non-dopaminergic pathway from/via MSt may be critical for exerting foraging effort.

Unexpectedly, 6-OHDA lesion did not suppress single running distance, suggesting that dopamine release in MSt does not affect the foraging effort. On the other hand, nonselective lesion of the dorsal MSt suppressed the foraging effort. The descending projection from MSt to midbrain dopaminergic nuclei (Bálint et al. 2011), but not the ascending dopaminergic projection from midbrain to MSt, may be involved in the behavioral control of effort output for reward.

The present result is inconsistent with earlier studies (Salamone et al. 1991, 1994), which report effort-aversion following 6-OHDA lesion to the rat ventral striatum (homologous to the chick MSt). In these studies, alternative decision paradigm was

adopted, in which rats were examined in prospective choices between less-preferred food reward following low effort and high-preferred food following high effort. This discrepancy may be due to the difference of experimental paradigm. In the present study, on the other hand, we presented the subjects with no explicit options for food. Instead, subjects just ran for and left from feeders, and foraged in these two feeders. Amount of 6-OHDA injected may also be important. Salamone et al. (1994) injected a larger amount (12.5 μg 6-OHDA) per injection site, while it was 3 μg 6-OHDA in the present experiment. Furthermore, these studies were accomplished in rats, and our study was in chicks.

It is also possible to argue that the nidopallium, rather than the MSt, is involved in the control of foraging effort, because the electrolytic lesion in MSt extended to nidopallium in most subjects (Figure III-4b). We suppose that we can disregard this possibility, because histological examination of a subject whose single running distance was not suppressed showed a broader lesion ranging from Wulst to nidopallium (Figure III-4c, A9.4); The dorsal part of MSt however remained intact in this subject.

3.4.2 Social facilitation may boost action value via the lateral dopaminergic pathway.

Functional and anatomical dissociation of medial and lateral dopaminergic pathway is well-known in mammals (O'Doherty et al. 2004, Balleine & O'Doherty 2012). From the viewpoint of reinforcement learning, "actor/critic model" (Sutton & Barto 1998) is widely adopted to explain the function of the neural circuits in basal ganglia (Montague et al. 1996, 2004). The medial pathway is assumed to be a "critic", and it monitors and evaluates the present state of rewards. On the other hand, the lateral

pathway is referred to as an “actor”, and it evaluates the value of executed actions. Although it is argued that such a dichotomy is too simple (Morris et al. 2006), converging evidences support that the lateral (SNc-dorsal striatum) pathway carries action-related value, but not the value of just current state (Reynolds et al. 2001, Samejima et al. 2005, Morris et al. 2006). We may argue, based on the results of present study in chicks, that social facilitation boosts the action value. Future electrophysiological study may provide evidence for this hypothesis. Actually, a recent study in primates revealed that some neurons in the dorsal striatum (assumed to be homologous to the avian LSt) encode social action and reward, although it remains unknown as to whether the action value was socially elevated or not (Báez-Mendoza et al. 2013).

3.4.3 SNr, rather than SNc, may be involved in the socially-facilitated foraging effort.

In this study, it remains still unclear as to whether substantia nigra pars compacta (SNc) or reticulata (SNr) is critical for social facilitation. SNr receives afferent inputs from dorsal striatum (or LSt) directly and indirectly. The direct input decreases, and the indirect input via subthalamic nucleus increases the firing rate in SNr neurons (Alexander & Crutcher 1990 for mammals; Reiner 2002 for birds). As SNc neurons do, SNr neurons also encode the value of reward cue, and it is assumed that the SNr neurons also exert direct control on actions (Bryden et al. 2011, Yasuda et al. 2012). Considering that (1) majority of SNr neurons are GABAergic (Fahn & Côté 1968, Okada et al. 1971, Veenman & Reiner 1994, Sun et al. 2005; also note that TH-positive cell bodies are also found in SNr, Figure III-5a), and that (2) the increase-type neurons

outnumber the decreasing-type neurons (Gulley et al. 2002), SNr lesion is supposed to cause excessive motor output, or behavioral disinhibition. Actually, with respect to eye movement in monkey, $GABA_A$ antagonist infused to SNr induces involuntary saccades and inability to maintain visual fixation (Hikosaka & Wurtz 1985). On the other hand, in our present study, comparable involuntary movements were not found in chicks with SN lesion. In order to further examine if SNr is a critical node of the social facilitation circuit, we must accomplish a selective lesion or inactivation of SNr while sparing the surrounding SNc. Selective inactivation of indirect pathway; i.e. inactivation of subthalamic nucleus, may be also useful.

3.5 Figures

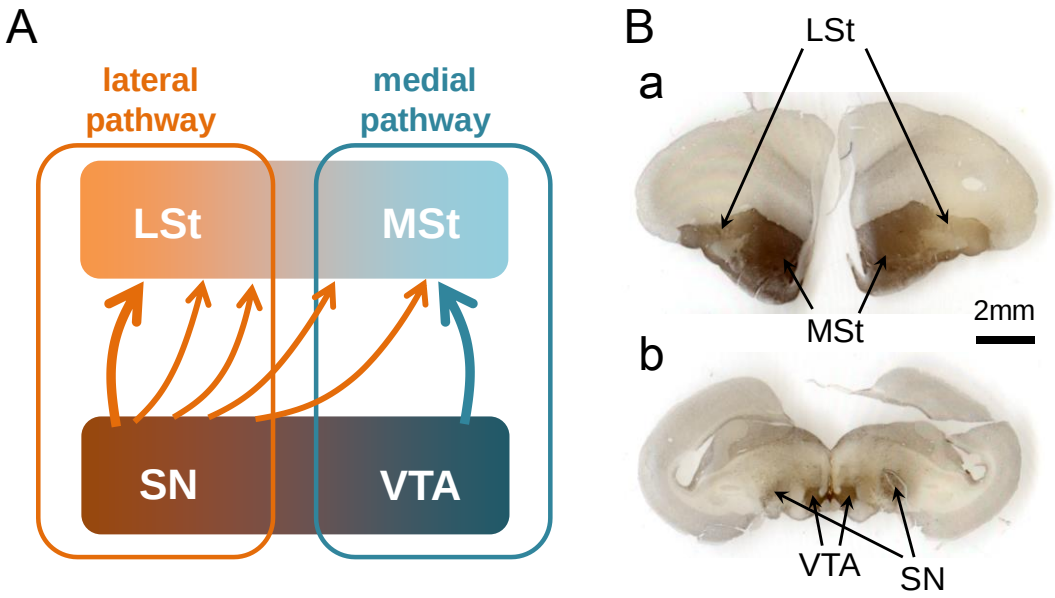


Figure III-1. (A) Schematic chart of anatomical construction of two pathways from midbrain to striatum. VTA dopaminergic neurons project to MSt, while no projection to LSt is found. On the other hand, SN dopaminergic neurons massively project to LSt, as well as to MSt. (B) Photographs of tyrosine hydroxylase-stained dopamine fibers and cell bodies in telencephalon (a) and midbrain (b) sections from an intact chick. Abbreviations; VTA, ventral tegmental area; MSt, medial striatum; SN, substantia nigra; LSt, lateral striatum.

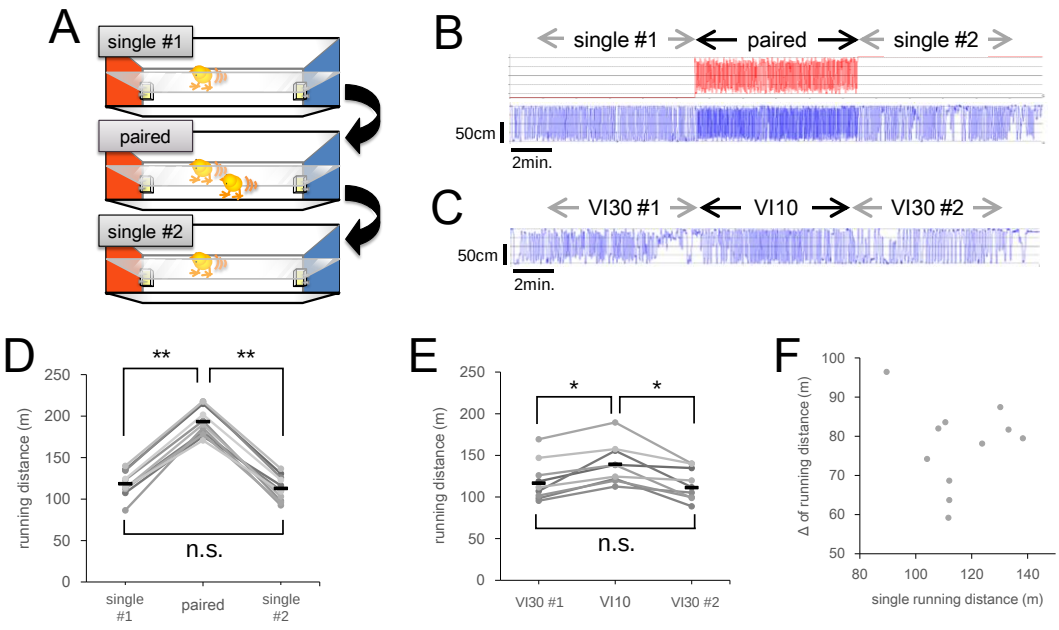


Figure III-2. Shuttles in I-shaped maze by sham chicks.

(A) Two-lane I-shaped maze equipped with a pair of terminal feeders. Chicks and feeders were separated by a transparent wall. Terminal walls of the maze were colored red and blue. Each feeders supplied 90 grains of millet according to a variable (10-20 seconds; average 15 seconds) interval schedule. At first subjects ran in single (upper). After 30 food deliveries from each feeders, a companion was introduced and ran in pairs (middle). After additional 30 food deliveries, the companion was removed and subjects ran in single again (lower). Each phase lasted for ca. 8 min.

(B) Representative running trajectories of a pair of chicks in a test. Subject chick is indicated by blue and companion is red. Note that there is no time lag between change of the phases and change of the pace of running by the subject.

(C) Representative running trajectory during a continuous series of VI30, VI10, and VI30 phases. Changes in running were instantaneous, but they were slower compared to the effect of putting/removing a companion (Figure III-2B).

(D) Running distance of all of the sham chicks (n=11). Although the session was

repeated 4 times in pre-operative session and 4 times in post-operative session, the graph indicates only the average result of the 4 sessions of after-operation. Running distance was significantly increased by pairing compared to both of single phases (pairwise comparisons using Wilcoxon signed rank test with Holm correction for multiple comparisons; p=0.0029). There was no significant difference between two single phases (p>0.05).

(E) Running distance of sham (n=9 of 11; 2 were not recorded) when the rate of feeding had been changed. In the VI10 (variable interval, average 10 seconds) phase, feeding rate is 3 times as high as that in the VI30 (variable interval, average 30 seconds) phases. Running distance was significantly increased in VI10 phase compared to both of VI30 phases (pairwise comparisons using Wilcoxon signed rank test with Holm correction for multiple comparisons; p=0.0120). There was no significant difference between two single phases (p>0.05). Note that running distance did not tripled when the feeding rate tripled.

(F) Scatter plot of average of single running distance (x axis) and Δ of running distance (y axis). Δ of running distance is defined as the difference between paired- minus average of single running distances. No significant correlation was observed (p>0.05).

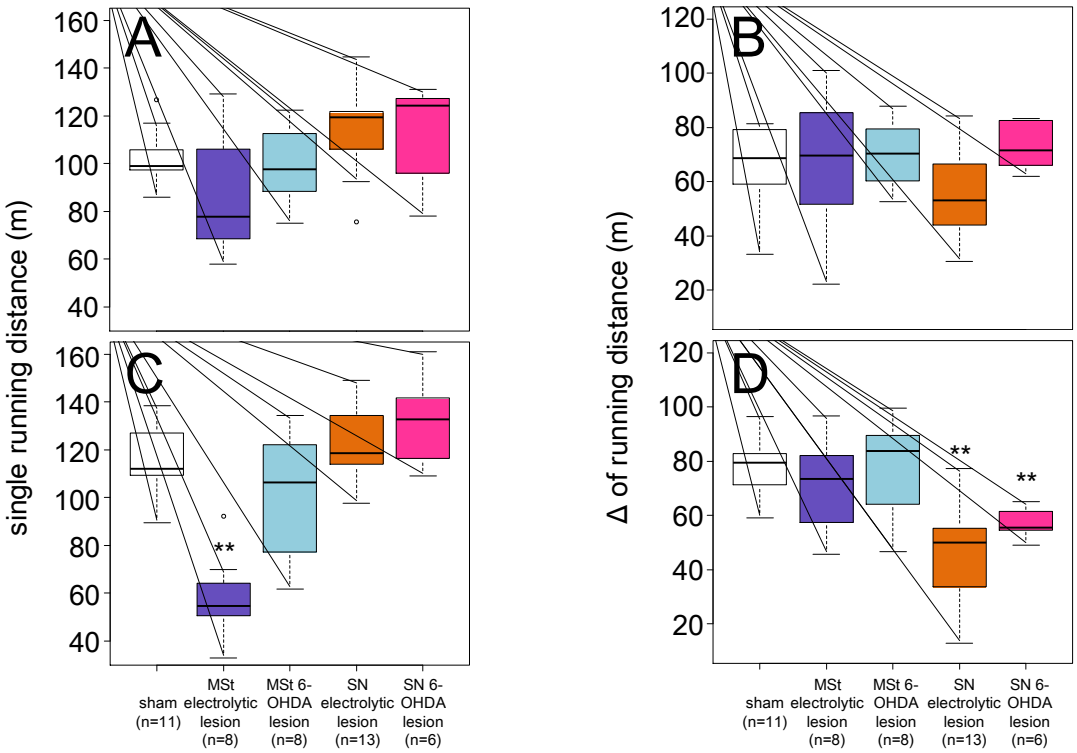


Figure III-3. Single running distance (A, pre-operative; C, post-operative) and Δ of running distance (B, pre-operative; D, post-operative). X axis indicates a sham group and 4 experimental groups. The top and bottom edge of boxes indicate the upper- and lower-quartile of each groups, respectively. The whiskers indicate the most extreme data point which is no more than 1.5 times the interquartile range from the box. Open circles indicate outliers. Kruskal-Wallis test revealed significant difference across groups in both single running distance and Δ of running distance for post-operation. For pre-operative data, no significant difference across groups were observed. Post-hoc Steel's multiple comparison test revealed sigpnificant difference between sham-MSt electrolytic lesion (p=0.0015) for single running distance, and between sham-SN electric lesion (p=0.0011) and sham-SN 6-OHDA lesion (p=0.0097) groups. Asterisks indicates significant difference compared to the sham group.

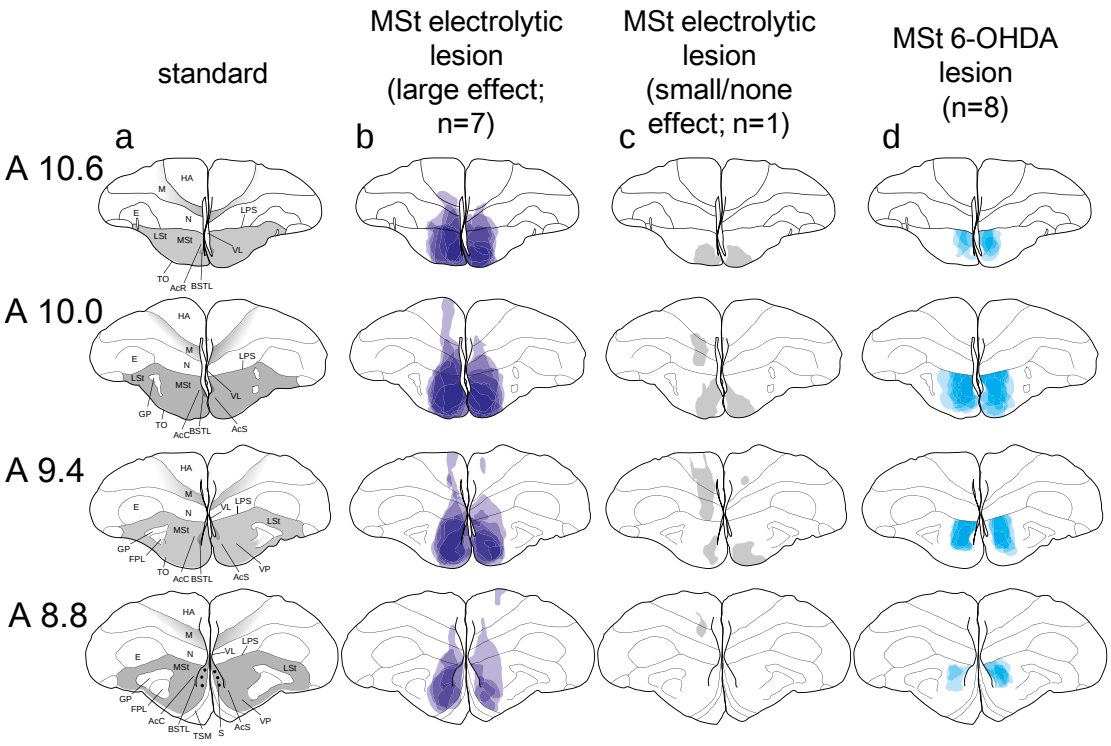


Figure III-4. Histological reconstruction of lesions in MSt. Lesioned areas are superimposed on frontal sections of the telencepharon. The coordinates (A8.2–A11.8) follow the stereotaxic atlas of Kuenzel and Masson (1988). Column "standard" (a) indicates the schematic depiction of TH-positive region of an intact brain. Black dots (see A 8.8) indicate TH-positive cell bodies, and dark areas indicate TH-positive terminals or other structures. Electrolytic lesioned chicks were divided into two categories according to single running distance; large effect (b; n=7) and small/none effect (c; n=1). Subjects whose single running distance were below 87.8 fell into large effect. Abbreviations of brain regions are based on Balint et al (2011).

Abbreviations; AcR, rostral pole of nucleus accumbens; AcC, Nucleus accumbens, core; AcS Nucleus accumbens, shell; 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; S, septum; TO, Olfactory tubercule; TSM, tractus septopallio-mesencephalicus; VL, Lateral ventricle; VP, ventral pallidum.

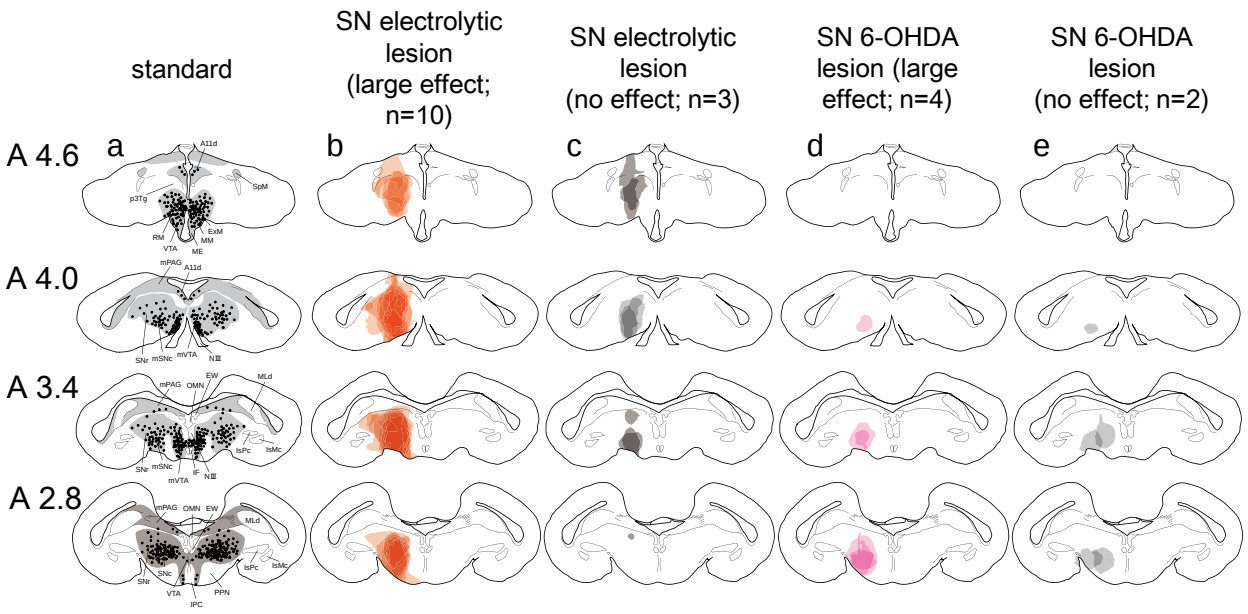


Figure III-5. Histological reconstruction of lesions in SN. Lesioned areas are superimposed on frontal sections of the midbrain. The coordinates (A4.6–A2.8) follow the stereotaxic atlas of Kuenzel and Masson (1988). Column (a) indicates the schematic depiction of TH-positive region of an intact midbrain. Lesioned areas are separately shown in both electrolytic-lesioned (b, c) and 6-OHDA-lesioned (d, e) groups, according to Δ of running distance. If average Δ was below 56.7, the subject was fallen into "large effect" (electrolytic lesion: b, 6-OHDA lesiom: e). Note that mechanically-lesioned site is shown in the sections of SN 6-OHDA lesion group (d, e).

Abbreviations; A11d, A11 dopamine cells, dorsal part; A11v, A11 dopamine cells, ventral 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 nucleus; RM, Retromammillary area; SNc, Substantia nigra, compact part; SNr, Substantia nigra, reticular part; SpM, nucleus spiriformis medialis; VTA, ventral tegmental area.

ACKNOWLEDGEMENT

First and foremost I would like to express my sincerest gratitude to my supervisor Professor Toshiya Matsushima. He has spent so much time and effort for teaching me. He enthusiastically taught me not only essential knowledge, techniques for experiments and how to write a paper, but also what science is. My six years in Matsushima laboratory will be an honorable epoch in my life.

I am also most grateful to Professors Makoto Mizunami, Professor Tatsuya Kameda and Dr. Masayo Soma for giving me critical comments and suggestions as members of the dissertation committee. I am also grateful to Professor Masakazu Takahata and Dr. Hiroto Ogawa for their helpful comments. I appreciate Professor Harumitsu Morohashi for giving me an opportunity to discuss about my study with researchers working in human science.

I owe deep gratitude to the members of Matsushima laboratory. Especially I express my gratitude to Dr. Hidetoshi Amita (now at National Institute of Health) for drawing my attention to social facilitation, and for frequent, stimulating and helpful discussions. I also much appreciate Shohei Matsunami (now at Fuji Xerox) for teaching me behavioral pharmacological techniques. I thank Dr. Ai Kawamori (now at The Graduate University for Advanced Studies) for her statistical advises. Thanks are also due to Momoko Miura, Chentao Wen, Ahmet Kerim Uysal and Kaname Fukuoka for providing me with helpful ideas for this study.

I sincerely appreciate to Dr. Takeshi Izumi, who was kind enough to offer me anti-TH antibody. I am also appreciate Dr. Kazuhiro Wada for offering me an immunohistochemistry protocol for avian brain. I thank a lot to Chihiro Mori, Shin

Hayase, Dr. Haruhito Horita and Dr. Eszter Bálint for giving me generous advices about immunohistochemistry procedure in detail. Furthermore I thank Takashi Katoh for making refined apparatuses.

I acknowledge the financial support of Japan Society for the Promotion of Science (JSPS). I was honored to be a JSPS fellow from 2012 to 2014 (#24006407). Grant and salary was not also financial, but also mental support for me to continue this study.

Finally I greatly appreciate my parents, who have raised me curiosity about the world, encouraged me to pursuit my scientific interest and supported me faithfully.

It is only the devoted efforts of all people I mention here that have made my study possible. Thanks are due to all people who were concerned with my thesis study.

REFERENCES

Agetsuma N. (1998) Simulation of patch use by monkeys using operant conditioning. *Journal of Ethology* **16**: 49–55.

Alexander, G. and Crutcher, M. (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in Neurosciences***13**: 266–271.

Allport F. H. (1920) The influence of the group upon association and thought. *Journal of Experimental Psychology* **3**: 159-182.

Amita H., Kawamori A. and Matsushima T. (2010) Social influences of competition on impulsive choices in domestic chicks. *Biology Letters* **6**: 183–186.

Amita H. and Matsushima T. (2011) Instantaneous and Cumulative Influences of Competition on Impulsive Choices in Domestic Chicks. *Frontiers in Neuroscience* **5**: 101.

Andrew, R., Johnston, A., Robins, A. and Rogers, L. (2004) Light experience and the development of behavioural lateralisation in chicks. II. Choice of familiar versus unfamiliar model social partner. *Behavioural Brain Research* **155**: 67–76.

Aoki N., Suzuki R., Izawa E.-I., Csillag A., and Matsushima T. (2006a) Localized lesions of ventral striatum, but not arcopallium, enhanced impulsiveness in choices based on anticipated spatial proximity of food rewards in domestic chicks. *Behavural Brain Research* **168**: 1-12.

Aoki N., Csillag A., and Matsushima T. (2006b) Localized lesions of arcopallium intermedium of the lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary choice task. *European Journal of Neuroscience* **24**: 2314-2326.

Báez-Mendoza, R., Harris, C. and Schultz, W. (2013) Activity of striatal neurons reflects social action and own reward. *Proceedings of the National Academy of Sciences of the United States of America* **110**: 16634–16639.

Bálint E. and Csillag A. (2007) Nucleus accumbens subregions: hodological and immunohistochemical study in the domestic chick (*Gallus domesticus*). *Cell & Tissue Research* **327**: 221–230.

Bálint, E., Kitka, T., Zachar, G., Adám, A., Hemmings, H. and Csillag, A. (2004) Abundance and location of DARPP-32 in striato-tegmental circuits of domestic chicks. *Journal of Chemical Neuroanatomy* **28**: 27–36.

Bálint E., Mezey S., Csillag A. (2011) Efferent Connections of Nucleus Accumbens Subdivisions of the Domestic Chicken (*Gallus domesticus*): An Anterograde Pathway Tracing Study. *Journal of Comparative Neurology* **519**: 2922–2953.

Balleine, B. and O'Doherty, J. (2010) Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology* **35**: 48–69.

Barnard C. J. and Sibly R. M. (1981) Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Animal Behaviour,* **29**: 543-550.

Bélisle M. (1998) Foraging group size: Models and a test with jaegers kleptoparasiting terns. *Ecology* **79**: 1922-1938.

Bendesky A., Tsunozaki M., Rockman M., Kruglyak L. and Bargmann C. (2011) Catecholamine receptor polymorphisms affect decision-making in C. elegans. *Nature* **472**:313–318.

Bizot J, Thiebot M, Le Bihan C, Soubrie P and Simon P. (1988) Effects of imipramine-like drugs and serotonin uptake blockers on delay of reward in rats. Possible

implication in the behavioral mechanism of action of antidepressants. *Journal of Pharmacology and Experimental Therapeutics* **246**:1144–1151.

Bizot J. C., Le Bihan C., Puech A. J., Hamon M. and Thiébot M. H. (1999) Serotonin and tolerance to delay of reward in rats. *Psychopharmacology* **146**: 400–412.

Blascovich, J., Mendes, W. B., Hunter, S. B. and Salomon, K. (1999) Social "facilitation" as challenge and threat. *Journal of Personality and Social Psychology* **77**: 68-77.

Brocco, M., Dekeyne, A., Veiga, S., Girardon, S. and Millan, M. J. (2002) Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake. A pharmacological characterization of diverse classes of antidepressant agents. *Pharmacology, Biochemistry & Behavior* **71**: 667–680.

Bryden, D., Johnson, E., Diao, X. and Roesch, M. (2011) Impact of expected value on neural activity in rat substantia nigra pars reticulata. *The European Journal of Neuroscience* **33**: 2308–2317.

Caraco, T., Martindale, S. and Whittam, T.S. (1980) An empirical demonstration of risk-sensitive foraging preferences. *Animal Behaviour* **28**: 820-830.

Cardinal, R., Pennicott, D., Sugathapala, C., Robbins, T. and Everitt, B. (2001) Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* **292**: 2499–2501.

Charnov, E. (1976a) Optimal foraging, the marginal value theorem. *Theoretical Population Biology* **9**: 129–136.

Charnov, E. L. (1976b) Optimal foraging: attack strategy of a mantid. *American Naturalist* **110**: 141-151.

Charrier, D. and Thiébot, M. (1996) Effects of psychotropic drugs on rat responding in

an operant paradigm involving choice between delayed reinforcers. *Pharmacology, Biochemistry & Behavior* **54**: 149–157.

Chen, S. C. (1937) Social Modification of the Activity of Ants in Nest-Building. *Physiological Zoölogy* **10**: 420-436.

Coull, J. T., Cheng, R. K. and Meck W. H. (2011) Neuroanatomical and neurochemical substrates of timing. *Neuropsychopharmacology* **36**: 3–25.

Curio, E. (1976) *The Ethology of Predation*. New York: Springer-Verlag.

Deng, C. and Rogers, L. J. (2002) Social recognition and approach in the chick: lateralization and effect of visual experience. *Animal Behaviour* **63**: 697–706.

Denk, F., Walton, M. E., Jennings, K. A., Sharp, T., Rushworth, M. F. and Bannerman, D. M. (2005) Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. *Psychopharmacology (Berlin)* **179**: 587-596.

Doya, K. (2008) Modulators of decision making. *Nature Neuroscience* **11**: 410–416.

Durstewitz, D., Kröner, S. and Güntürkün, O. (1999) The dopaminergic innervation of the avian telencephalon. *Progress in Neurobiology* **59**: 161–195.

Evenden, J. and Ryan, C. (1996) The pharmacology of impulsive behavior in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology* **128**: 161–170.

Fahn, S., and Ccote, L. J. (1968) Regional distribution of γ-aminobutyric acid (GABA) in brain of the rhesus monkey. *Journal of Neurochemistry*, **15**: 209–213.

Floresco, S. B. and Ghods-Sharifi, S. (2007) Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cerebral Cortex* **17**: 251-260.

Floresco, S. B., St. Onge J. R., Ghods-Sharifi S., and Winstanley C. A. (2008a). Cortico-

limbic-striatal circuits subserving different forms of cost- benefit decision making. *Cognitive, Affective, & Behavioral Neuroscience* **8**: 375-389.

Floresco, S. B., Tse, M. T. L., and Ghods-Sharifi, S. (2008b) Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. *Neuropsychopharmacology* **33**: 1966-1979.

Foster, W. A. and Treherne, J. E. (1981) Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature* **293**: 466-467.

Gates, M. F. and Allee, W. C. (1933) Conditioned behavior of isolated and grouped cockroaches on a simple maze. *Journal Comparative Psychology* **15**: 331-358.

Geyer, M. A. (1995) Serotonergic functions in arousal and motor activity. *Behavioural Brain Research* **73**: 31–35.

Giraldeau, L.-A. and Caraco, T. (2000) *Social foraging theory*. Princeton: Princeton University Press.

Glickman, S. E., Zabel, C. J., Yoerg, S. I., Weldele, M. L., Drea, C. M. and Frank, L. G. (1997) Social Facilitation, Affiliation, and Dominance in the Social Life of Spotted Hyenas. *Annals of the New York Academy of Sciences* **807**: 175-184.

Gulley, J. M., Kosobud, A. E. and Rebec, G. V. (2002) Behavior-related modulation of substantia nigra pars reticulata neurons in rats performing a conditioned reinforcement task. *Neuroscience* **111**: 337–349.

Haber, S., Fudge, J. and McFarland, N. (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. *Journal of Neuroscience* **20**: 2369–2382.

Hamilton, W. D. (1971) Geometry for the selfish herd. *Journal of Theoretical Biology*. **31**: 295-311.

Hayashi, I., Ono, Y. and Matsushima, T. (2001) Visual cues for suppressing the isolation-induced distress calls in newly hatched quail chicks. *Zoological Science* **18**: 1065-1071.

Hayden, B, Pearson, J and Platt, M. (2011) Neuronal basis of sequential foraging decisions in a patchy environment. *Nature Neuroscience* **14**: 933–939.

Hikosaka, O. and Wurtz, R. (1985) Modification of saccadic eye movements by GABA-related substances. II. Effects of muscimol in monkey substantia nigra pars reticulata. *Journal of Neurophysiology* **53**: 292–308.

Ho, M. Y., Al-Zahrani, S. S. A., Velazquez Martinez, D., Lopez Cabrera, M., Bradshaw, C. and Szabadi, E. (1996) Effects of desipramine and fluvoxamine on timing behavior investigated with the fixed-interval peak procedure and the interval bisection task. *Psychopharmacology* **125**: 274–284.

Ho, M.Y., Velázquez-Martínez, D., Bradshaw, C. and Szabadi, E. (2002) 5-Hydroxytryptamine and interval timing behavior. *Pharmacology, Biochemistry & Behavior* **71**: 773–785.

Izawa, E.-I., Zachar, G., Yanagihara, S. & Matsushima, T. (2003) Localized lesion of caudal part of lobus parolfactorius caused impulsive choice in the domestic chick: evolutionarily conserved function of ventral striatum. *The Journal of Neuroscience* **23**: 1894–1902.

Izawa, E.-I., Aoki, N. and Matsushima, T. (2005) Neural correlates of the proximity and quantity of anticipated food rewards in the ventral striatum of domestic chicks. *European Journal of Neuroscience* **22**: 1502–1512.

Jonsson, G. (1980) Chemical neurotoxins as denervation tools in neurobiology. *Annual Reviews of Neuroscience* **3**: 169–187.

Kacelnik, A. (1984) Central place foraging in starlings (Sturnus vulgaris). I. Patch residence time. *Journal of Animal Ecology* **53**: 283–299.

Kasuya, E. (1982) Central place water collection in a Japanese paper wasp, Polistes chinensis antennalis. *Animal Behaviour* **30**: 1010–1014.

Kawamori, A. and Matsushima, T. (2010) Subjective value of risky foods for individual domestic chicks: a hierarchical Bayesian model. *Animal Cognition* **13**: 431-441.

Koops, M. and Giraldeau, L.-A. (1996) Producer-scrounger foraging games in starlings: a test of mean-maximizing and risk-minimizing foraging models. *Animal Behaviour* **51**: 773-783.

Kuenzel, W. J. and Masson, M. (1988) *A Stereotaxic Atlas of the Brain of the Chick (Gallus Domesticus)*. Johns Hopkins University Press, Baltimore.

Larsen, M., Elfving, B. and Wiborg, O. (2004) The chicken serotonin transporter discriminates between serotonin-selective reuptake inhibitors. A species-scanning mutagenesis study. *Journal of Biological Chemistry* **279**: 42147–42156.

MacDonald, C. J. and Meck, W. H. (2005) Differential effects of clozapine and haloperidol on interval timing in the supraseconds range. *Psychopharmacology* 2005;182: 232–244.

Manns, M. and Güntürkün, O. (2003) Light experience induces differential asymmetry pattern of GABA- and parvalbumin-positive cells in the pigeon's visual midbrain. *Journal of Chemical Neuroanatomy* **25**: 249–259.

Matsushima, T., Kawamori, A. and Bem-Sojka, T. (2008) Neuro-economics in chicks: foraging choices based on amount, delay and cost. *Brain Research Bulletin* **76**: 245–252.

Matsushima, T., Izawa, E.-I., Aoki, N. and Yanagihara, S. (2003) The mind through

chick eyes: Memory, cognition and anticipation. *Zoological Science* **20**: 395-408.

Mazur, J. E. (1981) Optimization theory fails to predict performance of pigeons in a two-response situation. *Science* **214**: 823–825.

Meck, W. H. and Benson, A. M. (2002) Dissecting the brain's internal clock: how frontal-striatal circuitry keeps time and shifts attention. *Brain and Cognition* **48**: 195–211.

Mezey, S. and Csillag, A. (2002) Selective striatal connections of midbrain dopaminergic nuclei in the chick (Gallus domesticus). *Cell and Tissue Research* **308**: 35–46.

Metzger, M., Toledo, C. and Braun, K. (2002) Serotonergic innervation of the telencephalon in the domestic chick. *Brain Research Bulletin* **57**: 547–551.

Miyazaki, K., Miyazaki, K. W. and Doya, K. (2011a) Activation of dorsal raphe serotonin neurons underlies waiting for delayed rewards. *Journal of Neuroscience* **31**: 469–479.

Miyazaki, K. W., Miyazaki, K. and Doya, K. (2011b) Activation of central serotonergic system during work for delayed rewards. *European Journal of Neuroscience* **33**: 153–160.

Montague, P., Dayan, P. and Sejnowski T. J. (1996) A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *Journal of Neuroscience* **76**: 1936–1947.

Montague, P., Hyman, S. and Cohen, J. (2004) Computational roles for dopamine in behavioural control. *Nature* **431**: 760–767.

Moons, L., D'Hondt, E., Pijcke, K. and Vandesande, F. (1995) Noradrenergic system in the chicken brain: immunocytochemical study with antibodies to noradrenaline

and dopamine-beta-hydroxylase. *Journal of Comparative Neurology* **360**: 331–348.

Morris, G., Nevet, A., Arkadir, D., Vaadia, E. and Bergman, H. (2006) Midbrain dopamine neurons encode decisions for future action. *Nature Neuroscience* **9**: 1057–1063.

Mobini, S., Chiang, T. J., Ho, M. Y., Bradshaw, C. M. and Szabadi, E. (2000) Effects of central 5-hydroxytryptamine depletion on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology (Berlin)* **152**: 390–397.

Navailles, S. and De Deurwaerdère, P. (2011) Presynaptic control of serotonin on striatal dopamine function. *Psychopharmacology (Berlin)* **213**: 213–242.

O'Doherty, J., Dayan, P., Schultz, J., Deichmann, R., Friston, K. and Dolan, R. (2004) Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science* **304**: 452–454.

Ogura, Y. and Matsushima, T. (2011) Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks. *Frontiers in Neuroscience* **5**: 91.

Okada, Y., Nitsch-Hassler, C., Kim, J. S., Bak, I. J. and Hassler, R. (1971) Role of γ-aminobutyric acid (GABA) in the extrapyramidal motor system. I. Regional distribution of GABA in rabbit, rat, guinea pig and baboon CNS. *Experimental Brain Research* **13**: 514-518.

Owens, M. J., Knight, D. L., Nemeroff, C. B. (2001) Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. *Biological Psychiatry* **50**: 345–350.

R Development Core Team (2010) R version 2.12.0.: a language and environment for

statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.r-project.org.

Reiner, A. (2002) Functional circuitry of the avian basal ganglia: implications for basal ganglia organization in stem amniotes. *Brain Research Bulletin* **57**: 513–528.

Reiner, A., Perkel, D., Bruce, L., Butler, A., Csillag, A., Kuenzel, W., Medina, L., Paxinos, G., Shimizu, T., Striedter, G., Wild, M., Ball, G., Durand, S., Güntürkün, O., Lee, D., Mello, C., Powers, A., White, S., Hough, G., Kubikova, L., Smulders, T., Wada, K., Dugas-Ford, J., Husband, S., Yamamoto, K., Yu, J., Siang, C., Jarvis, E. and Gütürkün, O. (2004) Revised nomenclature for avian telencephalon and some related brainstem nuclei. *Journal of Comparative Neurology* **473**: 377–414.

Reynolds, J. N., Hyland, B. I., Wickens, J. R. (2001) A cellular mechanism of reward-related learning. *Nature* **413**: 67–70.

Rosvold, H. E., Mirsky, A. F., and Pribram, K. H. (1954) Influence of amygdalectomy on social behavior in monkeys. *Journal of Comparative and Physiological Psychology* **47**: 173-178.

Rudebeck, P. H., Walton, M. E., Smyth, A. N., Bannerman, D. M., and Rushworth, M. F. (2006a) Separate neural pathways process different decision costs. *Nature Neuroscience* **9**, 1161-1168.

Rudebeck, P. H., Buckley, M. J., Walton, M. E., and Rushworth, M. F. S. (2006b) A role for the macaque anterior cingulate gyrus in social valuation. *Science* **313**: 1310-1312.

Salamone, J. D., Steinpreis, R. E., McCullough, L. D., Smith, P., Grebel, D., and Mahan, K. (1991) Haloperidol and nucleus accumbens dopamine depletion

suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology* **104**: 515–521.

Salamone, J., Cousins, M. and Bucher, S. (1994) Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioural Brain Research* **65**: 221–229.

Salamone, J., Correa, M., Farrar, A. and Mingote, S. (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology* **191**: 461–482.

Samejima, K., Ueda, Y., Doya, K. and Kimura, M. (2005) Representation of action-specific reward values in the striatum. *Science* **310**: 1337–1340.

Schultz, W. (1998) Predictive reward signal of dopamine neurons. *Journal of Neurophysiology* **80**: 1–27.

Schultz, W. (2007) Behavioral dopamine signals. *Trends in neurosciences* **30**: 203–210.

Stephens, D. W. and Krebs, J. R. (1986) *Foraging theory*. New Jersey: Princeton University Press.

Stephens, D. W. and Anderson, D. (2001) The adaptive value of preference for immediacy: when shortsighted rules have farsighted consequences. *Behavioral Ecology* **12**: 330–339.

Strauss, B. (2002) Social facilitation in motor tasks: a review of research and theory. *Psychology of Sport and Exercise* **3**: 237–256.

Sun, Z., Wang, H., Laverghetta, A., Yamamoto, K. and Reiner, A. (2005) The distribution and cellular localization of glutamic acid decarboxylase-65 (GAD65) mRNA in the forebrain and midbrain of domestic chick. *Journal of Chemical*

Neuroanatomy **29**: 265–281.

Sutton, R. S. and Barto, A. G. (1998) *Reinforcement Learning: An Introduction*. MIT Press, Cambridge, MA.

Tolman, C. W. and Wilson, G. F. (1965) Social feeding in domestic chicks. *Animal Behaviour* **13**: 134-142.

Tolman, C. W. (1967) The varieties of social stimulation in the feeding behaviour of domestic chicks. *Behaviour* **30**: 275-286.

Triplett, N. (1898) The dynamogenic factors in pacemaking and competition. *Americal Journal of Psychology* **9**: 507-533.

Veenman, C.L. and Reiner, A. (1994) The distribution of GABA-containing perikarya, fibers, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. *Journal of Comparative Neurology* **339**: 209–250.

Walton, M., Croxson, P., Rushworth, M. and Bannerman, D. (2005) The mesocortical dopamine projection to anterior cingulate cortex plays no role in guiding effort-related decisions. *Behavioral Neuroscience* **119**: 323–328.

Walton, M. E., Kennerley, S. W., Bannerman, D. M., Phillips, P. E. and Rushworth, M. F. (2006) Weighing up the benefits of work: behavioral and neural analyses of effort-related decision making. *Neural Network* **19**: 1302-1314.

Walton, M., Groves, J., Jennings, K., Croxson, P., Sharp, T., Rushworth, M. and Bannerman, D. (2009) Comparing the role of the anterior cingulate cortex and 6-hydroxydopamine nucleus accumbens lesions on operant effort-based decision making. *European Journal of Neuroscience* **29**: 1678–1691.

Waselus, M., Galvez, J., Valentino, R. and Van Bockstaele, E. (2006) Differential

projections of dorsal raphe nucleus neurons to the lateral septum and striatum. *Journal of Chemical Neuroanatomy* **31**: 233–242.

Wogar, M. A., Bradshaw, C. and Szabadi, E. (1993) Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. *Psychopharmacology* **111**: 239–243.

Wolff, M. and Leander, J. (2002) Selective serotonin reuptake inhibitors decrease impulsive behavior as measured by an adjusting delay procedure in the pigeon. *Neuropsychopharmacology* **27**: 421–429.

Yadid, G., Pacak, K., Kopin, I. J., Goldstein, D.S. (1994) Endogenous serotonin stimulates striatal dopamine release in conscious rats. *Journal of Pharmacology and Experimental Therapeutics* **270**: 1158–1165.

Yanagihara, S., Izawa, E.-I., Koga, K. and Matsushima, T. (2001) Reward-related neuronal activities in basal ganglia of domestic chicks. *Neuroreport* **12**: 1431–1435.

Yasuda, M., Yamamoto, S. and Hikosaka, O. (2012) Robust representation of stable object values in the oculomotor Basal Ganglia. *The Journal of Neuroscience* **32**: 16917–16932.

Zajonc R. B. (1965) Social facilitation. *Science* **149**: 269-274.

Zeier, H. and Karten, H. (1971) The archistriatum of the pigeon: organization of afferent and efferent connections. *Brain Research* **31**: 313–326.

Zhou, F.M., Liang, Y., Salas, R., Zhang, L., De Biasi, M., Dani, J. (2005) Corelease of dopamine and serotonin from striatal dopamine terminals. *Neuron* **46**: 65–74.

LIST OF PUBLICATIONS

1. Yukiko Ogura and Toshiya Matsushima:

"Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks"

Frontiers in Neuroscience, Vol. 5, No. 91, pp. 1-8 (2011)

2. Shohei Matsunami, Yukiko Ogura, Hidetoshi Amita, Takeshi Izumi, Mitsuhiro Yoshioka and Toshiya Matsushima:

"Behavioural and pharmacological effects of fluvoxamine on decision-making in food patches and the inter-temporal choices of domestic chicks"

Behavioural Brain Research, Vol. 233, No. 2, pp. 577-586 (2012)

frontiers in
NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 21 July 2011
doi: 10.3389/fnins.2011.00091

Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks

Yukiko Ogura*[1] *and Toshiya Matsushima*[2]*

[1] *Laboratory for Animal Behavior and Intelligence, Graduate School of Life Science, Hokkaido University, Sapporo, Japan*
[2] *Faculty of Science, Laboratory for Animal Behavior and Intelligence, Department of Biology, Hokkaido University, Sapporo, Japan*

Edited by:
Marijn Van Wingerden, Heinrich-Heine University Duesseldorf, Germany

Reviewed by:
Ruud Van Den Bos, Utrecht University, Netherlands
Marietta Dindo, Zoo Atlanta, USA

****Correspondence:***
Toshiya Matsushima, Faculty of Science, Laboratory for Animal Behavior and Intelligence, Department of Biology, Hokkaido University, Kita 10, Nishi 8, Kita-ku, Sapporo 060-0810, Japan.
e-mail: matusima@sci.hokudai.ac.jp

Social influences on foraging efforts were examined in domestic chicks by investigating the frequency of runs made to feeders and the amount of pecking to gain food. Single or paired chicks foraged in an I-shaped maze equipped with a millet feeder on each end, that distributed one or two grains at variable intervals. Regardless of when the grain(s) were dispensed, chicks ran back and forth between the feeders. Analyses of their movement patterns revealed: (1) running patterns were not directly synchronized with the dispensing of grain(s), (2) running distance was longer in paired chicks than in single chicks, (3) paired chicks partially synchronized their runs between feeders, and (4) social effects were immediate but cumulative after repeated blocks. We further examined the social effects on running by dividing the I-maze into two parallel lanes separated by a transparent wall, so that *kleptoparasitic* interference of food did not occur. Again, the chicks increased their running speed and were even more synchronized with their partner's movements, indicating that food competition alone was not responsible for increased foraging effort. The number of pecks to get grains was also assessed under conditions where the food tray was gradually replaced, from an easy one to more difficult ones. When tested in the separated I-maze, paired chicks pecked more in the difficult food situation without increase in the number of gained grains. Results suggest that (i) social facilitation leads to increased foraging efforts and (ii) the presence of a conspecific is alone may lead to enhanced foraging efforts in chicks. These findings are discussed in terms of possible ecological background of social facilitation.

Keywords: work, cost, handling, consumption, competition, social foraging, kleptoparasitism

INTRODUCTION

Social facilitation, which results in an enhancement of behavioral performance or an increase in work investment when an individual is in the presence of one or more conspecifics, has been widely reported in a variety of animals including humans (Zajonc, 1965 for a review), e.g., ants building a nest (Chen, 1937), cockroaches running in mazes for food (Gates and Allee, 1933), hyenas in drinking behavior (Glickman et al., 1997), and humans engaged in physical work (Triplett, 1898) or mental work (Allport, 1920), leading to recent report on physiological characteristics of the facilitation in cardiovascular responses (Blascovich et al., 1999). While many diverse taxa exhibit socially facilitated behavior, surprisingly little is known about the ecological contexts in which social facilitation occurs.

From an ecological standpoint, social interference by conspecifics is considered an important factor in influencing an individual's foraging strategies. Classical foraging theory typically focuses on the perspective of a single forager (Charnov, 1976); however this neglects the overall interactions that occur in group-living animals. A more recent perspective, coined the "Social Foraging Theory," suggests that not only individual decision-making, but also conspecific behavior, influences the outcome of an individual's foraging success (Giraldeau and Caraco, 2000). For example, Parasitic Jaegers (*Stercorarius parasiticus*) will forage in larger groups when engaging in risky behavior such as attacking and steeling fish from Common Terns (*Sterna hirundo*), even though they could gain more food by foraging in smaller groups or by themselves (Bélisle, 1998). Bélisle (1998) argued that the risk of starving must also be included in foraging theory in addition to such things as net/gross intake rate or efficiency, since group formation is expected to reduce variance of the food-encounter rate.

In behavioral studies using chicks, Tolman and Wilson (1965) reported that paired chicks consumed a larger amount of food than did isolated chicks only when the chicks had been deprived of food. However, their study failed to show conclusive results on the effects of social facilitation on rates of pecking (Tolman, 1967). Tolman and Wilson (1965) also did not control the amount of food chicks could consume, thus it was uncertain whether the increased consumption was a result of the amount of food available rather than mere social facilitation. In our study, we tried to know whether social facilitation could improve individual pay-off [i.e., rate of net gain = (benefit − work cost)/time], or otherwise other currency (e.g., low probability of starvation; Caraco et al., 1980) should be considered.

In the present study, we examined the influence of social facilitation in 1 to 2-week-old domestic chicks (*Gallus domesticus*). By strictly controlling the amount of food delivered, we examined whether foraging competition (i.e., reduction of gain by interference of other individuals) may socially facilitate an increase in the amount of foraging efforts in chicks. Chicks provide a unique opportunity

for studying the neuroscience of decision-making in relation to behavioral ecology and economics (Matsushima et al., 2003, 2008 for reviews), because we can quantitatively control feeding conditions and potential energy budgets in experiments. Furthermore, chicks are precocial animals that begin to forage independently as soon as they hatch, and so individual development can be controlled as well. In our study, we investigated two foraging behaviors, running, and pecking, in order to assess whether foraging efforts would increase under specific social conditions (i.e., social facilitation). Running to, or approaching food, and pecking at, or handling, food have already been shown to have distinct neural substrates involved (ventral striatum/nucleus accumbens and arcopallium, respectively; Matsushima et al., 2008). For example, lesions of the ventral striatum enhanced choices of small/immediate reward against large/distant alternative (Izawa et al., 2003; Aoki et al., 2006a). Similarly, lesions of the arcopallium caused chicks to choose the small/easy reward more frequently than the large/costly alternative (Aoki et al., 2006b). It is therefore possible that social facilitation can occur differently in these two aspects of foraging effort.

MATERIALS AND METHODS

SUBJECTS

All 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, chicks were sacrificed in carbon dioxide according to the guidelines.

A total of 99 male domestic chicks (*G. domesticus*, White Leghorn strains) were used. New hatchlings (post-hatch day 1: presumed hatching day) were obtained from a local supplier (Hokuren Central Hatchery, Iwamizawa, Hokkaido, Japan). Chicks were paired and housed in transparent plastic cages (15 cm × 28 cm × 12 cm) under white lighting (12L: 12D; light period starting at 08:00) and thermo-controlled at ca. 30°C. Pairs of chicks in the same cages were trained and tested in the same conditions.

Two types of food were given, grains of millet and chick mash food. The total amount of food per day was kept at a certain level so that (1) the body weight of chicks gradually increased and (2) the chicks actively consumed food during experiments. From post-hatch day 2, chicks were fed mash food. The amounts of mash food were 1 g (post-hatch days 2–5), 1.5 g (days 6 and 7), and 3 g (from day 8). From post-hatch day 3, grains of millet were added. The amounts of grains (per chick per day) were 1 g (day 3), 2 g (day 4), 3 g (day 5), 2.5 g (days 6 and 7), and 2 g (from day 8). Until day 3, all chicks were communally fed. From day 4, chicks were allocated to a communally fed condition or solitarily fed condition depending on experiments: varied among groups in Experiment 1 and solitarily in Experiments 2 and 3. In the groups of solitarily fed chicks, each individual was fed in a cage that was visually separated by a black plastic wall, so that chicks did not see the other chicks eating food; these chicks were communally housed except when the daily diet was given.

APPARATUS

In Experiment 1, an I-shaped maze equipped with one-lane (1-lane maze; 12 cm in width, 88 cm in length and 30 cm in height) was used (**Figure 1A**). The maze was equipped with a pair of terminal feeders.

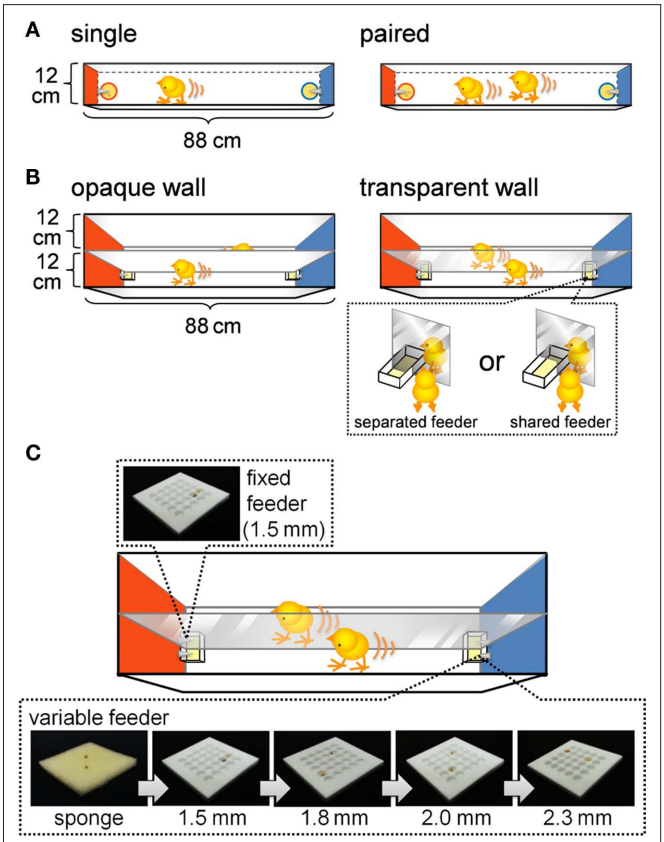


FIGURE 1 | Experimental apparatus for examining foraging efforts, running distance (A,B), and number of pecks (C). (A) One-lane I-shaped maze equipped with a pair of terminal feeders. Terminal walls of the maze were colored red and blue. The feeders supplied grains of millet according to a variable interval schedule. One grain was supplied at one time in the single-chick condition (left), and two grains were supplied at one time in the paired-chicks condition (right). Note that the paired chicks were competing over food, whereas the single chicks were not. **(B)** Two-lane I-shaped maze. Chicks and feeders were separated by an opaque (left) or a transparent (right) wall. In the transparent wall condition, the feeders were either separated or shared. Note that the paired chicks in the shared feeder condition were competing for food, whereas chicks in the other condition did not. **(C)** The 2-lane maze was equipped with a fixed feeder and a variable feeder. The food tray of the fixed feeder was made of a plastic plate with 25 holes (5 × 5), each 1.5 mm deep. The food tray of the variable feeder was either a sponge or plastic plates of variable depth (ranging from 1.5 to 2.3 mm). Supplied grains tumbled into the holes, making it difficult for chicks to obtain the grains. The deeper the holes were, the more difficult it was for chicks to obtain the grains. Trays of the variable feeder were sequentially replaced in an order from the easy sponge to the more difficult plates every ca. 3 min. See text for details.

Walls at the terminals were colored red (left) or blue (right). Each feeder supplied a grain of millet food at variable intervals. Plastic Petri dishes (5.5 cm in diameter to a depth of 1.5 cm) were used as food trays, and the floor of each dish was covered with sponge.

In Experiment 2, an I-shaped maze equipped with two lanes (2-lane maze; 25 cm in width, 88 cm in length, and 40 cm in height) was used (**Figure 1B**). These two lanes had the same width as that of the 1-lane maze used in Experiment 1 but were separated by a transparent acryl board in one groups of chicks. In another group, in order to visually separate the two lanes, opaque white cardboard was

attached to both sides of the acrylic board so that the chicks could not see each other. On each terminal feeder, two food trays were placed in adjacent positions over the separation board. Rectangle-shaped food trays (3 cm in width, 4 cm in length, and 2 cm in depth) with sponge on the floor were use. In the shared feeder condition, a 4-cm-wide window was opened on each terminal end of the separation board, and the chicks in both lanes shared the food supplied to a food tray (6 cm in width, 4 cm in length, and 2 cm in depth) placed at the center.

In Experiment 3, the same 2-lane maze as that in Experiment 2 was used after a slight modification to the food trays (**Figure 1C**). Square food trays (width and length of 3.6 cm depth of 1.8 cm) were used with two different types of floor coverage: one with sponge and the other with acrylic plates with 25 holes in the surface (aligned in 5×5, 4 mm in diameter). Four different types of acryl plates were used with different depths of the holes: 1.5, 1.8, 2.0, and 2.3 mm. Food trays were manually replaced, and a single replacement took ca. 10 s.

In all experiments, in order to prevent chicks to associate the feeder sound with food reward, sounds of electric motors were replayed at variable intervals from instruments placed around the apparatus; the mean interval was set at 2.5 s, uniformly distributed from 1.5 to 3.5 s. The apparatus was placed in a dark room kept at ca. 25–30°C and illuminated by four 60 W white light bulbs placed above the runway and feeders. Timing of grain delivery and the noise sound were controlled by microrobots (RCX, LEGO Mindstorms). Behavior of the chicks was recorded by a video recorder (DCR-SR65, Sony, Japan) and color CCD cameras (250 k pixels with NTSC output), and the recordings were stored for offline analysis.

BEHAVIORAL PROCEDURES

In Experiments 1–3, chicks were initially habituated to the experimental maze according to a common procedure for two successive days (pre-1 and pre-2) on post-hatch days 6–15. For habituation, paired chicks (housed in the same cages) were placed on the midway part of the maze in which some food (ca. 100 grains) was given in advance. After the chicks had consumed the food, feeders started to deliver grains by the same procedure as that used in each experiment. Two successive habituation sessions (ca. 20 min in total) were given per day for each of pre-1 and pre-2 except for Experiment 3, in which one long session of habituation (ca. 20 min) was given instead. The experimental data were obtained after the habituation.

Experiment 1: effects of paired foraging on approaching food, an inter-group comparison in the 1-lane maze

Effects of paired foraging in the maze and the cage were examined in regards to subjects' distance and synchrony during running. Running distance was used to calculate the rate of approaching food at either end of the I-maze, where an increase in distance corresponds to increased running during the experimental condition.

"Paired in the maze" means that chicks foraged in pairs at the test, whereas "paired in the cage" means that the chicks foraged in pairs in the housing cage, in which main diet (mixture of mash food and millet) was supplied (**Figure 3A**).Chicks were thus divided into four conditions: (1) paired in the maze/paired in the cage, (2) paired in the maze/single in the cage, (3) single in the maze/paired in the cage, (4) single in the maze/single in the cage.

After the habituation (pre-1 and pre-2), experiments were conducted for five consecutive days (days 1–5) and chicks received one test session per day. In each of the sessions, after a short habituation period in the maze (i.e., 1 min after the chick had consumed all of the food available, namely, 20 grains/chick placed in advance in each feeder), each of the two feeders supplied one grain at a time with variable intervals (mean interval of 15 s, uniformly distributed in a range of 10–20 s). In a single session, the programmed food delivery continued 30 times and thus lasted for ca. 8 min. The chicks were then left in the maze for an additional 2 min after they had consumed all of the grains delivered on the food trays, and the test session of the day was terminated.

In the I-shaped maze, the following two parameters were measured: (1) running distance (or how far the chick ran) and (2) synchrony index. Synchrony index was defined as the ratio of time in which both chicks stayed in the same side of the maze, shown as percentage of the total time recorded. The position of each chick's head was automatically and unequivocally given by computer-based video analysis, as either being placed on the red or the blue side of the maze. When both chicks were always in the same end, the synchrony index was 1.0 (in-phase synchrony). When, on the other hand, chicks were in opposite ends, the index was 0.0 (anti-phase synchrony). When both chicks moved in a random and independent fashion, the index would show a chance level of 0.5 (asynchrony).

Experiment 2: effects of paired foraging on approaching food, an inter-group comparison in the 2-lane maze

In order to differentiate food competition from social facilitation by a nearby conspecific, we removed the factor of food competition by separating paired chicks with either a transparent or an opaque partition down the middle of the maze's track, thus creating two separate lanes (**Figure 1B**). After the habituation (i.e., paired foraging in the maze on pre-1 and pre-2), similar to Experiment 1, test sessions were repeated five times, one session per day (days 1–5). Each of the two feeders supplied one grain at a time with variable intervals (mean interval of 15 s, uniformly distributed in a range of 10–20 s). In a single session, the programmed food delivery continued 30 times and thus lasted for ca. 8 min. The following two parameters were measured: (1) running distance and (2) synchrony index.

Effects of paired foraging on running distance, an intra-individual comparison

The immediate effect of paired foraging was examined in terms of running distance. A group of chicks (post-hatch days 12–14) were re-used after Experiments 1 and 2 (see above); chicks that had been solitary fed in both the maze and the cage were used. Immediately after each chick had been individually placed in the maze, both feeders delivered grain 10 times at variable intervals (mean interval of 15 s, uniformly distributed in a range of 10–20 s, 1 grain per delivery); this term is referred to as the *first "single" phase*. The companion chick was then introduced into the maze, and the feeders delivered twice as much grain (2 grains per delivery) 10 times; this term is referred to as the second *"paired" phase*. In the third phase, the companion chick was removed from the maze, and the subject chick received feeding another 10 times. Each phase lasted for ca. 3 min.

Experiment 3: effects of paired foraging on approaching and pecking at food, an inter-group comparison in the 2-lane maze

Effects of visual perception of the other chick on running (i.e., approaching food) and on food pecking (handling food) were examined in the 2-lane maze. The food tray difficulty (estimated on the basis of the number of pecks required for chicks to gain a certain amount of grain) was controlled by systematically changing the types of acryl plates used as the floor of the feeder (**Figure 1C**). After habituation (i.e., paired foraging in the maze on pre-1 and pre-2), similar to Experiments 1 and 2, test sessions were repeated three times, one session per day (days 1–3); chicks were left untested for 4 days between test days 1 and 2. Each of the two feeders supplied two grains at a time with variable intervals (mean interval of 30 s, uniformly distributed in a range of 20–40 s).

In the tests, the variable feeder initially had a sponge floor. When the variable feeder had delivered six times (12 grains), the sponge was replaced by an acryl plate with 1.5-mm-deep holes. The 1.5-mm plate was subsequently replaced by 1.8, 2.0, and 2.3 mm plates when the variable feeder had delivered 12 grains for each plate. Four CCD cameras were set just above the feeder to record pecking behavior of the chicks were video recorded. The following four parameters were measured: (1) number of pecks, (2) number of gained grains, (3) running distance and (4) velocity. Velocity (cm/s) was measured in the runway except for the areas near the feeders (<10 cm from the walls).

DATA ANALYSIS

Recording and analyzing approaching behavior

Experiments were videotaped and coded later at rate of 30 frames per second using a Handycam recorder. The Handycam was located directly above the I-maze during testing, providing an aerial view of the subjects and apparatus. Chicks were individually marked by a rectangular piece of fluorescent-colored tape (Yamato Co., Ltd., Japan) affixed to their heads. The position of the fluorescent markers was analyzed by using Move-tr/2D 7.0 software (Library Co., Japan) and the trajectories and running distances were thus calculated.

Statistical analysis of generalized linear mixed model

The following five types of behavioral parameters were analyzed by using R for the platform of statistic calculation: running distance, synchrony index, number of pecks, number of gained grains and velocity. See Appendix for details.

RESULTS

Once habituated, chicks began to actively run between the terminal feeders as soon as they were put in the maze, even without any visual cues. Furthermore, the runs were not in response to the timing of food delivery. Singly tested chicks stopped running within ca. 1 min of the final delivery of food items. We therefore assumed that running distance of the runs represented "approaching effort" and pecks at the feeders represented "handling effort," rather than reflexive responses to food, and examined food-approaching behaviors in a series of experiments.

EXPERIMENT 1: PAIRED FORAGING INCREASED RUNNING DISTANCE

Paired chicks ran more than single chicks did. **Figure 2A** shows representative running trajectories of single (top and second records) and paired chicks (third and fourth records) at tests. Runs by single chicks were irregular on day 1 (top record), but they were more regular and more active on day 5 (second record). Runs by paired chicks were highly synchronized on day 1 (third record), but they were unsynchronized on day 5 (fourth record). Superimposed trajectories (**Figure 2B**), however, showed that the runs were not in response to the timing of food delivery.

Paired foraging in the maze, but not in the cage, increased running distance and synchrony index. A comparison of running by the four groups of chicks (n = 10 in each groups) is shown in **Figure 3B**. Based on running distance, AICs were calculated for each of the eight models in which the variables of *day* (1–5), *maze* (paired or single), and *cage* (paired or single) were considered (See **Table A1** in Appendix for details). Of these models, the *day-maze* model yielded the smallest AIC. The *day-maze-cage* model yielded the same-AIC, but the *cage* term was not reliable for its coefficient (p = 0.197). Similarly, based on the synchrony index, AIC calculations revealed a facilitating effect of paired foraging in the maze, but

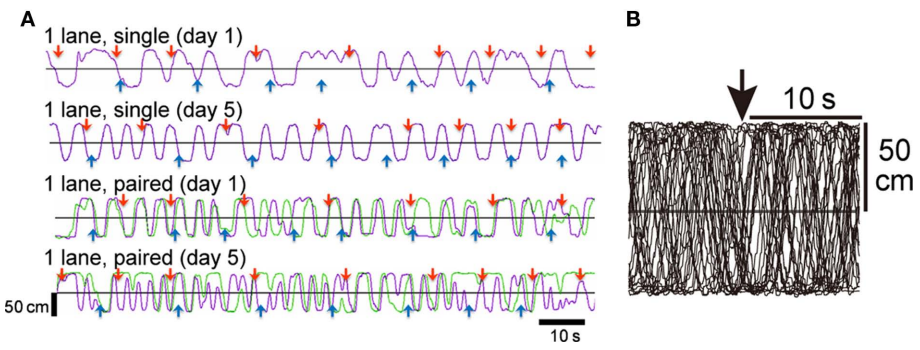


FIGURE 2 | Representative running trajectories in the 1-lane condition (Experiment 1). (A) Two minutes records of running trajectories. Purple and green lines represent the trajectory of individuals along the long axis of the maze; upward indicates the direction to the red feeder. Red and blue arrows indicate the time at which a grain(s) was delivered. The top and second records (1-lane, single) were obtained from the same chick on different days (days 1 and 5). The third and fourth records (1-lane, paired) were obtained from a pair of individuals. Comparison of the trajectories on day 1 (top vs. third) and day 5 (second vs. fourth) clearly shows that the paired chicks ran at a higher frequency. **(B)** Thirty superimposed trajectories aligned at the time of grain delivery from the red feeder (downward arrow at the top). An example obtained from a chick tested in the single condition in the maze. Note that the runs were not in response to the food delivery.

not those of paired foraging in the cage (**Table A2** in Appendix). We therefore concluded that the presence of other chicks and/or food competition among paired chicks could have caused the increase in approaching effort. It is not clear whether synchronization might be directly (and causally) linked to the increased effort.

EXPERIMENT 2: VISUAL PERCEPTION OF THE OTHER CHICK, BUT NOT FOOD COMPETITION, EXCESSIVELY INCREASED RUNNING DISTANCE

To reveal the cause of the increased running distance, we separated the maze into two lanes by a transparent/opaque wall (see **Figure 1B**), and we found that visual perception increased both running and synchrony indexes. **Figure 4** shows representative trajectories obtained from one pair in each group. When separated by an opaque wall, chicks in the 2-lane maze (therefore with no food competition) ran back and forth between feeders independently (top record in **Figure 4**). When visually interacting via a transparent wall, chicks ran back and forth in high synchronization (second record). Direct foraging competition at shared feeders (see **Figure 1B**, bottom) did not result in difference from when the transparent wall separating the feeders was present (compare second and bottom record in **Figure 4**). It is notable that a high degree of synchrony was maintained until day 5, in contrast to the unsynchronized running seen in Experiment 1 (see **Figure 2A**, fourth record, and **Figure 3B**, bottom).

Based on the running distance and synchrony index (**Figure 5**), AICs were calculated for eight models. As variables, *day* (1–5), *wall* (whether the wall was transparent or opaque), and *feeder* (whether the feeders were separated or shared) were considered (**Table A3** in Appendix). For running distance, the *day-wall* model yielded the smallest AIC (16191). The *day-wall-feeder* model gave rise to the second-smallest-AIC (16192), but the *feeder* term was not reliable for its coefficient ($p = 0.2921$). For synchrony index (**Table A4** in Appendix), the *day-wall* model yielded the smallest AIC, but the *day* term was not reliable for its coefficient ($p = 0.0844$); thus, the result is different from the synchrony index in Experiment 1, in which the coefficient of the *day* was negative. We therefore tentatively conclude that the running distance and synchrony index are not linearly linked. Increased effort is not brought about by changes in synchronized running.

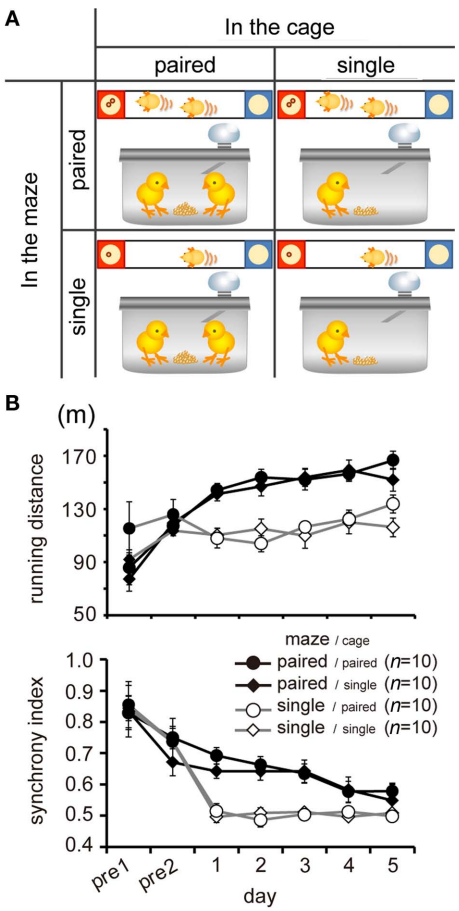


FIGURE 3 | Paired foraging in the maze, but not in the cage, influenced running (Experiment 1). (A) Chicks were divided into four experimental groups according to 2 × 2 block placement. "In the maze" means chicks were single or paired in the I-shaped maze during the tests, whereas "in the cage" means that chicks foraged in single or paired condition in their home cage. Note that chicks were housed in pairs in all conditions except foraging. **(B)** Means (±SEM) of running distance (upper) and synchrony index (lower) during feeding time (ca. 8 min) are plotted against the day of the experiment. Open and filled symbols denote paired and single foraging in the maze, whereas circles and rhombi denote paired and single foraging in the cage, respectively, in this and the following figures.

PAIRING IMMEDIATELY INCREASED RUNNING DISTANCE

In both Experiments 1 and 2, difference in running distance between the groups appeared from day 1 of the experiment. In order to determine whether paired foraging could immediately increase the approaching effort, we examined running distances by an intra-individual comparison. As shown in a typical example (**Figure 6A**), pairing immediately increased running. The average running distance for each condition (mean ± SEM, $n = 14$) is shown in **Figure 6B**. The Wilcoxon signed-rank test revealed a significant difference between paired phase and mean of the first and last phases ($T = 2$, p-value = 0.0003662).

EXPERIMENT 3: PAIRING INCREASED PECKS WITHOUT IMPROVED FOOD GAIN

In order to determine whether paired chicks also increased their handling effort (i.e., efforts to collect food), we examined rates of pecking for food by using a series of trays that modified the

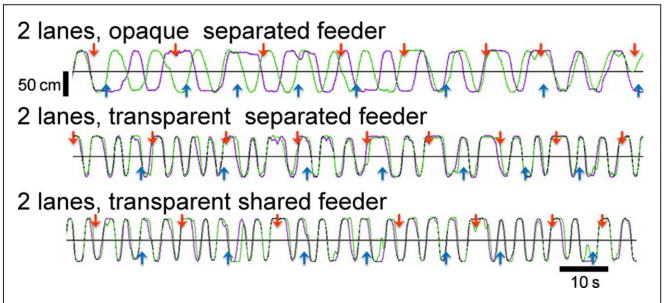


FIGURE 4 | Representative running trajectories in the 2-lane condition (Experiment 2). When separated by an opaque wall, running trajectories were not synchronized (top). When separated by a transparent wall, runs were more frequent and synchronized regardless of whether the feeder was separated (middle) or shared (bottom). All records were obtained on experimental day 5.

difficulty for food collection (**Figure 1C**). Paired chicks increased the number of pecks, particularly in the difficult food condition (in a range from 1.8 to 2.3 mm; **Figure 7A**). The number of grains decreased in accordance with increased difficulty of food tray, but the number of grains was not different between the single and paired groups (**Figure 7B**). Running distance was greater for paired chicks as found in Experiments 1 and 2, but the difference gradually diminished in the difficult food condition (**Figure 7C**). However, the velocity at which paired chicks ran remained consistently higher than in the single chicks (**Figure 7D**). The running distance of paired chicks decreased not because they ran slowly but because they stayed at the feeders for a longer time.

For all of the data shown in **Figures 7A–D**, AICs were calculated for five models in which the variables of *feeder* (food tray difficulty, five steps ranging from sponge to 2.3 mm), *maze* (paired or single), and their *interaction* were considered (**Tables A5–A8** in Appendix). For the number of pecks, the *feeder–maze-interaction* model yielded the smallest AIC (**Table A5** in Appendix), indicating that the effects of pairing emerged on handling effort only when the food was difficult to obtain. For the number of grains, on the other hand, the *maze* term was not included in the chosen model (**Table A6** in Appendix), indicating that the paired and single chicks had similar gains. For running distance, the *feeder–maze-interaction* model was chosen (**Table A7** in Appendix). The *interaction* term suggested that the difference between the paired and the single chicks were smaller for the more difficult food trays. For velocity, the *maze* model was chosen (**Table A8** in Appendix).

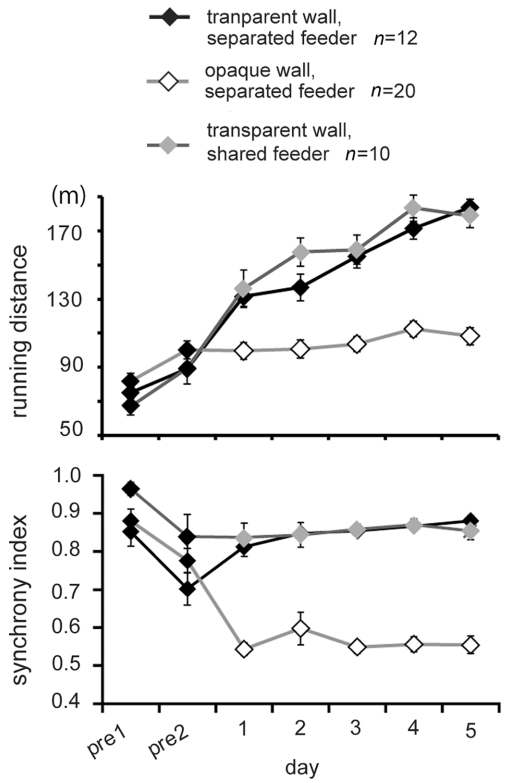


FIGURE 5 | Social interactions via the transparent wall caused a high degree of synchrony regardless of foraging competition (Experiment 2). Means (±SEM) of running distance (upper) and synchrony index (lower) are plotted against the day of the experiment. Open and filled symbols denote data in the opaque wall and the transparent wall, respectively. Gray symbols denote the data obtained from another group in which the feeders were shared and the wall was transparent.

DISCUSSION

ECOLOGICAL ACCOUNTS OF THE EXCESSIVE FORAGING EFFORTS

In this study, we found that visual perception of other individuals, rather than direct foraging competition, increased foraging efforts for approaching food (running distance) as well as for handling food (number of pecks). Increased foraging efforts in this study appear to be a result of social facilitation. Zajonc (1965) argued that social facilitation affects "dominant responses," meaning that dominant (or well-developed) action patterns are most likely to be socially enhanced. Both running and pecking are well-developed behaviors in actively foraging domestic chicks. It should be noted, however, that chicks never exhibit running or pecking behavior when food is not available (data not shown), indicating that direct inter-individual interactions alone failed to cause social facilitation. It should also be noted that the term "social facilitation" is a psychological label, never specifying its functions in terms of economics/ecology. The idea of social facilitation is therefore not mutually exclusive with the idea of work investment under competition.

Our results suggest that currencies (or value functions) other than the food benefit are critical in social facilitation, in accordance with social foraging theory. According to Koops and Giraldeau (1996), starlings adopt foraging tactics that minimize the probability of energetic shortfall rather than maximize mean intake rate (or the benefit). Chicks may also make use of other individuals'

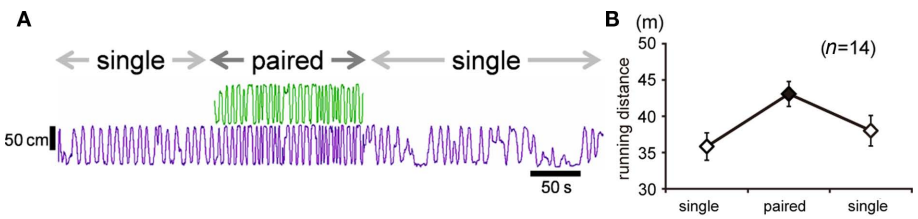


FIGURE 6 | The run was facilitated as soon as a companion chick was introduced (Experiments 1 and 2). (A) Representative running trajectories of a pair of chicks. Subject chick (purple) received 30 deliveries of food, divided into three phases. In the first phase, the subject was tested in single condition. In the second phase, a companion chick (green) was introduced into the maze. In the third phase, the subject was again tested in single condition. Each phase lasted for ca. 3 min. **(B)** Means (±SEM) of running distance recorded in the first and third phases of the single condition (open symbols) and in the second phase of the paired condition (filled symbol).

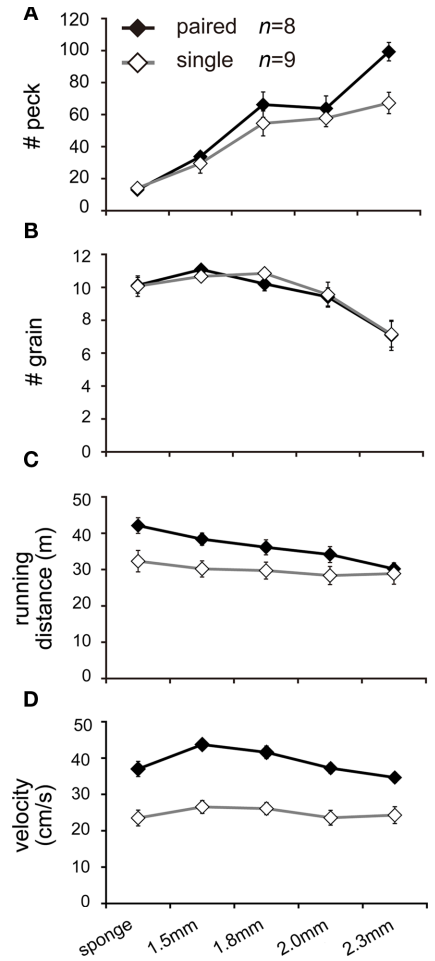


FIGURE 7 | Social interactions of pairing were also found in the number of pecks (handling effort), particularly in the difficult food condition (Experiment 3). Means (±SEM) of two groups of chicks [paired (filled symbols) and single conditions (open symbols)] are plotted against the phases of different food tray difficulty (from the sponge to the plate with deep holes). Each individual chick was repeatedly tested on three successive days, and the values were averaged to yield individual data. Number of pecks **(A)**, total gain of grains **(B)**, running distance **(C)**, and mean velocity **(D)** are plotted. Note that the chicks spent a shorter time for running but ran at higher velocity in the paired condition, even in the difficult condition.

information at food sites to avoid the risk of starvation, given that chicks have been shown to be risk-averse to food quantities (Kawamori and Matsushima, 2010). However, much remains to be discussed about whether excessive running and pecking was really "inefficient," since we were not able to calculate the energetic cost of running and pecking in terms of joules/calories. It is still possible that running and pecks are energetically very cheap and that the overt investments found in this study do not cause a negative energetic budget. Precise measurements of physical expenditures for running and pecking actions are needed.

Excessive foraging efforts found in this study might also lead to impulsive choices. Amita et al. (2010) reported that repeated experience of competitive foraging for a few days resulted in a higher level of choice impulsivity, though the chicks were solitarily tested in an inter-temporal choice paradigm. On the other hand, the impulsivity did not change instantaneously when the chicks were tested in competition (Amita and Matsushima, unpublished); the increase in impulsivity was observed even when there was no actual food competition, similar to our results. It remains to be determined whether the visual perception of competitive individuals directly caused the impulsivity or whether the excessive work investments secondarily caused the impulsivity. Further studies are required.

ECOLOGICAL ACCOUNTS OF SYNCHRONIZED RUNNING

The synchronized running observed in this study (Experiments 1 and 2) may be explained by "scramble kleptoparasitism" in behavioral ecology. Kleptoparasitism refers to parasitical exploitation of food that other foragers' efforts have made available (Giraldeau and Caraco, 2000). Of several variations, scramble kleptoparasitism specifically refers to the simultaneous exploitation of a sharable resource by multiple competitors with little or no aggression. This foraging behavior has also been called "social facilitation" (Curio, 1976). Barnard and Sibly (1981) regarded interactions between kleptoparasitically foraging individuals as "producer/scrounger" relationships, or a pair of alternative strategies.

It is possible that chicks running together acted as producer and scrounger, the one leading acted as a producer and the other following acted as a scrounger. In this study, we found that some individuals behaved predominantly as followers; e.g., in the bottom record of **Figure 4**, the purple-colored chick tended to follow the green-colored chick. However, the tactics were not always fixed, and chicks often changed their position in reference to the other. Foraging competition did not influence the synchrony of running (Experiment 2, **Figure 5**, bottom), indicating that chicks did scramble kleptoparasitism due to some innate or developmental factors, rather than to immediate competition over food. In accordance with this, we often observed that a chick was attracted to its pair mate and stayed at the feeder, even though the subject chick had already gained a grain at that feeder (e.g., bottom traces in **Figure 4**). To reveal the direct cause of synchronization, more elaborate analysis of running synchrony is needed.

The synchronization may also be an adaptive response to predation risk. Hamilton (1971) points out that predation could lead to the evolution of gregarious behavior by considering that predators habitually approach from outside of the herd. Furthermore, gregariousness itself "dilutes" predation risk for any particular individuals (Foster and Treherne, 1981). Highly synchronized runs observed in our study may have resulted from the gregarious instincts in chicks. It is therefore quite interesting to examine if chicks under predation pressure could run in even higher level of synchronization.

IMPLICATIONS FOR NEURAL MECHANISMS OF SOCIAL FACILITATION

In the neuroscience of decision-making, relevant brain regions and neurotransmitters/neuromodulators critical for "effort cost" (such as pressing a lever or climbing a mesh barrier to obtain food pellets) have been intensively explored (Walton et al., 2006; Floresco et al., 2008a). Several brain regions are reported to cause a work cost aversion, meaning a bias away from the costly option (e.g., climbing mesh barrier) to obtain a larger food; i.e., the anterior cingulate cortex (Rudebeck et al., 2006a), the neural pathways between the anterior cingulate cortex and amygdala, and the amygdala itself (Floresco and Ghods-Sharifi, 2007).

Systemic administration of dopamine antagonists (Denk et al., 2005; Floresco et al., 2008b) also induced the same effect. Since the anterior cingulate cortex and amygdala are thought to be involved in social behaviors (Rosvold et al., 1954; Rudebeck et al., 2006b), these regions might play a critical role in social influences of behavior by conspecifics. However, no studies have so far integrated the neuroscience of economical decision-making and the behavioral ecology of social foraging. Our next goal is therefore to clarify the neural mechanisms that underlie the social influences on work investments.

CONCLUSION

When viewed from behavioral economics and ecology, social facilitation can be characterized by increased foraging efforts and synchronization among individuals. The facilitation occurs immediately by visual perception of other individuals, rather than the accompanying food competition. The increased efforts occur not only in the approaching to food resource (running) but also in the handling food (pecking). Other factors than the gain rate should be considered, such as minimizing the starvation risk or adaptation to predation pressure.

ACKNOWLEDGMENTS

This study was supported by grant-in-aid for scientific research to Toshiya Matsushima from the Japanese Society for the Promotion of Sciences (#22570070), as well as that for Innovative Area and from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (The study on the neural dynamics for understanding communication in terms of complex hetero systems; #22120502). We would like to express our gratitude to anonymous referees, who patiently gave us detailed, generous, and critical comments on our initial manuscript.

REFERENCES

Allport, F. H. (1920). The influence of the group upon association and thought. *J. Exp. Psychol.* 3, 159–182.

Amita, H., Kawamori, A., and Matsushima, T. (2010). Social influences of competition on impulsive choices in domestic chicks. *Biol. Lett.* 6, 183–186.

Aoki, N., Suzuki, R., Izawa, E.-I., Csillag, A., and Matsushima, T. (2006a). Localized lesions of ventral striatum, but not arcopallium, enhanced impulsiveness in choices based on anticipated spatial proximity of food rewards in domestic chicks. *Behav. Brain. Res.* 168, 1–12.

Aoki, N., Csillag, A., and Matsushima, T. (2006b). Localized lesions of arcopallium intermedium of the lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary choice task. *Eur. J. Neurosci.* 24, 2314–2326.

Barnard, C. J., and Sibly, R. M. (1981). Producers and scroungers: a general model and its application to captive flocks of house sparrows. *Anim. Behav.* 29, 543–550.

Bélisle, M. (1998). Foraging group size: models and a test with jaegers kleptoparasitizing terns. *Ecology* 79, 1922–1938.

Blascovich, J., Mendes, W. B., Hunter, S. B., and Salomon, K. (1999). Social "facilitation" as challenge and threat. *J. Pers. Soc. Psychol.* 77, 68–77.

Caraco, T., Martindale, S., and Whittam, T. S. (1980). An empirical demonstration of risk-sensitive foraging preferences. *Anim. Behav.* 28, 820–830.

Charnov, E. L. (1976). Optimal foraging: attack strategy of a mantid. *Am. Nat.* 110, 141–151.

Chen, S. C. (1937). Social modification of the activity of ants in nest-building. *Physiol. Zoöl.* 10, 420–436.

Curio, E. (1976). *The Ethology of Predation.* New York: Springer-Verlag.

Denk, F., Walton, M. E., Jennings, K. A., Sharp, T., Rushworth, M. F., and Bannerman, D. M. (2005). Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. *Psychopharmacology (Berl)* 179, 587–596.

Floresco, S. B., and Ghods-Sharifi, S. (2007). Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb. Cortex* 17, 251–260.

Floresco, S. B., St. Onge, J. R., Ghods-Sharifi, S., and Winstanley, C. A. (2008a). Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. *Cogn. Affect. Behav. Neurosci.* 8, 375–389.

Floresco, S. B., Tse, M. T. L., and Ghods-Sharifi, S. (2008b). Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. *Neuropsychopharmacology* 33, 1966–1979.

Foster, W. A., and Treherne, J. E. (1981). Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature* 293, 466–467.

Gates, M. F., and Allee, W. C. (1933). Conditioned behavior of isolated and grouped cockroaches on a simple maze. *J. Comp. Psychol.* 15, 331–358.

Giraldeau, L.-A., and Caraco, T. (2000). *Social Foraging Theory*. Princeton: Princeton University Press.

Glickman, S. E., Zabel, C. J., Yoerg, S. I., Weldele, M. L., Drea, C. M., and Frank, L. G. (1997). Social facilitation, affiliation, and dominance in the social life of spotted hyenas. *Ann. N. Y. Acad. Sci.* 807, 175–184.

Hamilton, W. D. (1971). Geometry for the selfish herd. *J. Theor. Biol.* 31, 295–311.

Izawa, E.-I., Zachar, G., Yanagihara, S., and Matsushima, T. (2003). Localized lesion of caudal part of lobusparolfactorius caused impulsive choice in domestic chick: evolutionary conserved function of ventral striatum. *J. Neurosci.* 23, 1894–1902.

Kawamori, A., and Matsushima, T. (2010). Subjective value of risky foods for individual domestic chicks: a hierarchical Bayesian model. *Anim. Cogn.* 13, 431–441.

Koops, M., and Giraldeau, L.-A. (1996). Producer-scrounger foraging games in starlings: a test of mean-maximizing and risk-minimizing foraging models. *Anim. Behav.* 51, 773–783.

Matsushima, T., Izawa, E.-I., Aoki, N., and Yanagihara, S. (2003). The mind through chick eyes: memory, cognition and anticipation. *Zool. Sci.* 20, 395–408.

Matsushima, T., Kawamori, A., and Bem-Sojka, T. (2008). Neuro-economics in chicks: foraging choices based on amount, delay and cost. *Brain Res. Bull.* 76, 245–252.

Rosvold, H. E., Mirsky, A. F., and Pribram, K. H. (1954). Influence of amygdalectomy on social behavior in monkeys. *J. Comp. Physiol. Psychol.* 47, 173–178.

Rudebeck, P. H., Walton, M. E., Smyth, A. N., Bannerman, D. M., and Rushworth, M. F. (2006a). Separate neural pathways process different decision costs. *Nat. Neurosci.* 9, 1161–1168.

Rudebeck, P. H., Buckley, M. J., Walton, M. E., and Rushworth, M. F. S. (2006b). A role for the macaque anterior cingulate gyrus in social valuation. *Science* 313, 1310–1312.

Tolman, C. W. (1967). The varieties of social stimulation in the feeding behaviour of domestic chicks. *Behaviour* 30, 275–286.

Tolman, C. W., and Wilson, G. F. (1965). Social feeding in domestic chicks. *Anim. Behav.* 13, 134–142.

Triplett, N. (1898). The dynamogenic factors in pacemaking and competition. *Am. J. Psychol.* 9, 507–533.

Walton, M. E., Kennerley, S. W., Bannerman, D. M., Phillips, P. E., and Rushworth, M. F. (2006). Weighing up the benefits of work: behavioral and neural analyses of effort-related decision making. *Neural Netw.* 19, 1302–1314.

Zajonc, R. B. (1965). Social facilitation. *Science* 149, 269–274.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 April 2011; accepted: 03 July 2011; published online: 21 July 2011.

*Citation: Ogura Y and Matsushima T (2011) Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks. Front. Neurosci. **5**:91. doi: 10.3389/fnins.2011.00091*

This article was submitted to Frontiers in Decision Neuroscience, a specialty of Frontiers in Neuroscience.

APPENDIX

STATISTICAL ANALYSIS USING GENERALIZED LINEAR MIXED MODEL

In Experiment 1 and 2, we focused on running distance and total time in which both chicks stayed in the same end of the maze (i.e., numerator of synchrony index) as response variables. In Experiment 3, we focused on number of pecks, number of gained grains, running distance, and velocity as response variables.

We assumed a Poisson distribution for the error structure of the data of running distance, number of pecks, and number of gained grains, considering that they were all non-negative values. $\Lambda(X)$ (>0) was thus approximated by a Poisson function (log link function) as

$$\Lambda(X) = \exp(X) \tag{1}$$

On the other hand, we assumed a binomial distribution for the error structure of the data of total time in which both chicks stayed in the same end of the maze, since the time was calculated by the number of video frames and all frames were fallen into either "same end" or "different end" category. Synchrony index = $Q(X)$ ($\in [0, 1]$) was thus approximated by a logistic function (logit link function) as

$$Q(X) = 1/(1+\exp(-X)) \tag{2}$$

in which a predictor X was linearly given as a weighed sum of explanatory variables.

Experiment 1

$$X = \beta_0 + (\beta_1 + r_{is}) * \text{day} + \beta_2 * \text{maze} + \beta_3 * \text{cage} + r_{ii} \tag{3}$$

Day (variable = 1, 2, 3, 4, or 5) denotes experimental days. Coefficient β_1 indicates how the day contributes to running distance or synchrony index. A positive value of estimated β_1 thus suggests that the running distance increases as the days elapses.

Maze (categorical variable) denotes foraging condition in the maze (i.e., single or paired). Coefficient β_2 indicates how paired foraging in the maze contributes to the response variables. A negative value of estimated β_2 suggests that single chicks in the maze ran/synchronized less than paired chicks in the maze.

Cage (categorical variable) denotes foraging condition in the cage when the main diet food was supplied (i.e., single or paired). Coefficient β_2 indicates how paired foraging in the cage contributes to the response variables. A negative value of estimated β_2 suggests that runs by single chicks in the cage ran/synchronized less than paired chicks in the cage.

Experiment 2

$$X = \gamma_0 + (\gamma_1 + r_{is}) * \text{day} + \gamma_2 * \text{maze} + \gamma_3 * \text{feeder} + r_{ii} \tag{4}$$

Maze (categorical variable) denotes the type of wall in the maze (i.e., transparent or opaque). Coefficient γ_2 indicates how visual perception contributes to the response variables. A negative value of estimated γ_2 suggests that chicks mutually invisible ran/synchronized less than chicks mutually visible.

Feeder (categorical variable) denotes whether the feeders were separated or shared. Coefficient γ_3 indicates how the actual competition over food intake contributes to the response variables. A negative value of estimated γ_3 suggests that the actual competition over food intake decreased running distance/synchrony less than chicks with no competition.

Experiment 3

$$X = \delta_0 + \delta_1 * \text{maze} + (\delta_2 + r_{is}) * \text{feeder} + \delta_3 * \text{maze} * \text{feeder} + r_{ii} \tag{5}$$

Maze (categorical variable) denotes foraging condition in the maze (i.e., single or paired).

Feeder (numeric variable; 1, 2, 3, 4, and 5) denotes difficulty of food trays. Coefficient δ_2 indicates how increasing difficulty of food trays contributes to the response variables. A positive value of estimated δ_2 suggests that the more difficult the food tray was, the larger the response variable was.

Coefficient δ_3 indicates how *maze* and feeder interact.

The intercepts (β_0, γ_0, and δ_0) denote bias at the population level. The random intercept and the random slope (against day and feeder in Experiment 1, 2, and 3, respectively) for each individual (i) was denoted by r_{ii} and r_{is}, representing noise that was not experimentally controlled; Gaussian distribution with mean = 0 was assumed.

In Experiment 1, AICs were compared among eight models with different combination of parameters; (i) *null model* (β_0), (ii) *day model* (β_0, β_1), (iii) *maze model* (β_1, β_2), (iv) *cage model* (β_1, β_3), (v) *day-maze model* (β_0, β_1, β_2), (vi) *day-cage model* (β_0, β_1, β_3), (vii) *maze-cage model* (β_0, β_2, β_3) and (viii) *day-maze-cage model* (β_0, β_1, β_2, β_3).

Similarly in Experiment 2, AICs were compared among the following eight models; (i) *null model* (γ_0), (ii) *day model* (γ_0, γ_1), (iii) *maze model* (γ_0, γ_2), (iv) *feeder model* (γ_0, γ_3), (v) *day–maze model* (γ_0, γ_1, γ_2), (vi) *day–feeder model* (γ_0, γ_1, γ_3), (vii) *maze–feeder model* (γ_0, γ_1, γ_3), and (viii) *day–maze-feeder model* (γ_0, γ_1, γ_2, γ_3).

In Experiment 3, AICs were compared among the following five models; (i) *null model* (δ_0), (ii) *maze model* (δ_0, δ_1), (iii) *feeder model* (δ_1, δ_2), (iv) *maze–feeder model* (δ_0, δ_1, δ_2), and (v) *maze–feeder-and-interaction model* (δ_0, δ_1, δ_2, δ_3).

Most likely values of the intercepts and coefficients (β_0, β_1, β_2, β_3, γ_0, γ_1, γ_2, γ_3, and δ_0, δ_1, δ_2, δ_3) were estimated on the basis of the choice data by using R (version 2.12.0; R Development Core Team, 2010) and the lme4 package (version 0.999375-37; Bates and Sarkar, 2010). AICs were given as a sum of the deviance plus two times the number of parameters. The AICs and the parameter estimates are shown in **Tables A1–A8** in Appendix for Experiment 1, 2, and 3, respectively. For interpretations of the statistic computations, see the main text.

In all tables, models are sorted in the order of AICs. Hyphen means that the model does not include the parameter. Coefficients in parentheses represents that the 95% confidence interval (CI) of the estimate included 0. CI was not considered for the intercepts (β_0, γ_0, and δ_0).

REFERENCES

Bates, D. M., and Sarkar, D. (2010). *lme4: Linear Mixed-effects Models Using S4 classes, R Package Version 0.999375-37*. Available at: http://cran.r-project.org/web/packages/lme4/index.html

R Development Core Team. (2010). *R version 2.12.0.: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Table A1 | The *day–maze* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			β_0 (Intercept)	β_1 (Day)	β_2 (Maze)	β_3 (Cage)
1	[β_0, β_1, β_2]	14794	9.475460	0.033304	–0.347460	–
2	[β_0, β_1, β_2, β_3]	14794	9.442871	0.033308	–0.347421	(0.065203)
3	[β_0, β_2]	14812	9.47573	–	–0.34738	–
4	[β_0, β_2, β_3]	14813	9.44303	–	–0.34736	(0.06539)
5	[β_0, β_1]	14822	9.301773	0.033290	–	–
6	[β_0, β_1, β_3]	14823	9.269186	0.033299	–	(0.065212)
7	[β_0]	14841	9.30201	–	–	–
8	[β_0, β_3]	14842	9.26935	–	–	(0.06536)

AICs for running distance (Experiment 1). AICs and estimated coefficients of variables were calculated for 8 models designed for running distance in Experiment 1. The models are sorted in ascending order of AIC. Coefficients in parentheses represents that the 95% confidence interval (CI) of the estimate included 0. The model [β_0, β_1, β_2] indicates that both day and maze had significant effects, whereas the same-AIC. Model [β_0, β_1, β_2, β_3] indicates that cage was not reliable for its coefficient. Most-likely fitting formulas are indicated below:
Single foraging in the maze: $X = 9.128 + (0.033304 + r_{is}) \times day + r_{ii}$.
Paired foraging in the maze: $X = 9.475460 + (0.033304 + r_{is}) \times day + r_{ii}$.

Table A2 | The *day–maze* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			β_0 (Intercept)	β_1 (Day)	β_2 (Maze)	β_3 (Cage)
1	[β_0, β_1, β_2]	4834	0.85135	–0.05698	–0.83931	–
2	[β_0, β_1, β_2, β_3]	4835	0.89544	–0.05698	–0.83924	(–0.08825)
3	[β_0, β_2]	4839	0.85106	–	–0.83970	–
4	[β_0, β_2, β_3]	4840	0.89519	–	–0.83963	(–0.08833)
5	[β_0, β_1]	4862	0.43171	–0.05700	–	–
6	[β_0, β_1, β_3]	4864	0.47585	–0.05698	–	(–0.08837)
7	[β_0]	4867	0.4311	–	–	–
8	[β_0, β_3]	4869	0.47541	–	–	(–0.08846)

AICs for synchrony index (Experiment 1). AICs and estimated coefficients of variables were calculated for 8 models designed for synchrony index (proportion of the number of video flames in which chicks were in the same end of the maze) in Experiment 1. The model [β_0, β_1, β_2] indicates that both day and maze had significant effects, whereas the second-smallest-AIC model [β_0, β_1, β_2, β_3] indicates that cage was not reliable for its coefficient.
Single foraging in the maze: $X = 0.01204 + (-0.05698 + r_{is}) \times day + r_{ii}$.
Paired foraging in the maze: $X = 0.85135 + (-0.05698 + r_{is}) \times day + r_{ii}$.

Table A3 | The *day–maze* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			γ_0 (Intercept)	γ_1 (Day)	γ_2 (Maze)	γ_3 (Feeder)
1	$[\gamma_0, \gamma_1, \gamma_2]$	16191	9.320326	0.060972	–0.276690	–
2	$[\gamma_0, \gamma_1, \gamma_2, \gamma_3]$	16192	9.270098	0.060971	–0.226365	(0.110372)
3	$[\gamma_0, \gamma_1, \gamma_3]$	16196	9.128636	0.060968	–	0.251822
4	$[\gamma_0, \gamma_1]$	16201	9.188624	0.060951	–	–
5	$[\gamma_0, \gamma_2]$	16228	9.32036	–	–0.27610	–
6	$[\gamma_0, \gamma_2, \gamma_3]$	16229	9.27008	–	–0.22573	(0.11048)
7	$[\gamma_0, \gamma_3]$	16233	9.12902	–	–	0.25154
8	$[\gamma_0]$	16237	9.18896	–	–	–

AICs for running distance (Experiment 2). AICs and estimated coefficients of variables were calculated for 8 models designed for running distance in Experiment 2. The model $[\gamma_0, \gamma_1, \gamma_2]$ indicates that both day and maze had significant effects, whereas the second-smallest-AIC model $[\gamma_0, \gamma_1, \gamma_2, \gamma_3]$ indicates that feeder was not reliable for its coefficient.
Opaque wall: $X = 9.043636 + (0.060972 + r_{is}) \times day + r_{ii}$.
Transparent wall: $X = 9.320326 + (0.060972 + r_{is}) \times day + r_{ii}$.

Table A4 | The *day–maze* model yielded the smallest AIC, but the effect of *day* was not reliable for its coefficient.

	Models	AIC	Estimated coefficients of variables			
			γ_0 (Intercept)	γ_1 (Day)	γ_2 (Maze)	γ_3 (Feeder)
1	$[\gamma_0, \gamma_1, \gamma_2]$	14088	1.54956	(0.04025)	–1.29326	–
2	$[\gamma_0, \gamma_2]$	14089	1.5501	–	–1.2934	–
3	$[\gamma_0, \gamma_1, \gamma_2, \gamma_3]$	14089	1.42322	(0.04025)	–1.16667	(0.27757)
4	$[\gamma_0, \gamma_2, \gamma_3]$	14090	1.4236	–	–1.1667	(0.2782)
5	$[\gamma_0, \gamma_3]$	14105	0.6943	–	–	1.0073
6	$[\gamma_0, \gamma_1, \gamma_3]$	14105	0.69392	(0.04026)	–	1.00706
7	$[\gamma_0, \gamma_1]$	14110	0.93383	(0.04022)	–	–
8	$[\gamma_0]$	14111	0.9340	–	–	–

AICs for synchrony index (Experiment 2). AICs and estimated coefficients of variables were calculated for 8 models designed for synchrony index in Experiment 2. The model $[\gamma_0, \gamma_1, \gamma_2]$ indicates that day had insignificant effects.
Opaque wall: $X = 0.2567 + (0.04025 + r_{is}) \times day + r_{ii}$.
Transparent wall: $X = 1.5501 + (0.04025 + r_{is}) \times day + r_{ii}$.

Table A5 | The *maze–feeder-and-interaction* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			δ_0 (Intercept)	δ_1 (Maze)	δ_2 (Feeder)	δ_3 (Maze:feeder)
1	$[\delta_0, \delta_1, \delta_2, \delta_3]$	434.8	2.67886	(0.04795)	0.39100	–0.07668
2	$[\delta_0, \delta_2]$	437.7	2.69015	–	0.35387	–
3	$[\delta_0, \delta_1, \delta_2]$	439.4	2.74001	(–0.09332)	0.35381	–
4	$[\delta_0]$	490.3	2.6991	–	–	–
5	$[\delta_0, \delta_1]$	492.3	2.67413	(0.04772)	–	–

AICs for the number of pecks (Experiment 3). AICs and estimated coefficients of variables were calculated for 5 models designed for number of pecks in Experiment 3. The model $[\delta_0, \delta_1, \delta_2, \delta_3]$ indicates that maze per se had no effects in the absence of feeder.
Single: $X = 2.72681 + (0.31432 + r_{is}) \times feeder + r_{ii}$.
Paired: $X = 2.67886 + (0.39100 + r_{is}) \times feeder + r_{ii}$.

Table A6 | The *feeder* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			δ_0 (Intercept)	δ_1 (Maze)	δ_2 (Feeder)	δ_3 (Maze:feeder)
1	[δ_0, δ_2]	42.02	2.48704	–	–0.07750	
2	[δ_0, δ_1, δ_2]	44.01	2.483046	(0.007534)	–0.077502	
3	[δ_0, δ_1, δ_2, δ_3]	45.98	2.494080	(–0.013251)	–0.081384	(0.007306)
4	[δ_0]	49.77	2.26054	–	–	
5	[δ_0, δ_1]	51.76	2.25654	(0.00753)	–	

AICs for the number of gained grains (Experiment 3). AICs and estimated coefficients of variables were calculated for 5 models designed for total gain of grains in Experiment 3. The model [δ_0, δ_2] indicates that only feeder had significant effects.
$X = 2.48704 + (–0.07750 + r_{is}) \times feeder + r_{ii}$.

Table A7 | The *maze–feeder-interaction* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			δ_0 (Intercept)	δ_1 (Maze)	δ_2 (Feeder)	δ_3 (Maze:feeder)
1	[δ_0, δ_1, δ_2, δ_3]	1640	8.41344	–0.34862	–0.07784	0.04685
2	[δ_0, δ_1, δ_2]	1643	8.41253	–0.34666	–0.05309	–
3	[δ_0, δ_2]	1650	8.22898	–	–0.05308	–
4	[δ_0, δ_1]	1655	8.41218	–0.34806	–	–
5	[δ_0]	1662	8.22792	–	–	–

AICs for running distance (Experiment 3). AICs and estimated coefficients of variables were calculated for five models designed for running distance in Experiment 3. The model [δ_0, δ_1, δ_2, δ_3] indicates that maze, feeder and their interaction had significant effects.
Single: $X = 8.06482 + (–0.03099 + r_{is}) \times feeder + r_{ii}$.
Paired: $X = 8.41344 + (–0.07784 + r_{is}) \times feeder + r_{ii}$.

Table A8 | The *maze* model yielded the smallest AIC.

	Models	AIC	Estimated coefficients of variables			
			δ_0 (Intercept)	δ_1 (Maze)	δ_2 (Feeder)	δ_3 (Maze:feeder)
1	[δ_0, δ_1]	77.45	3.65249	–0.45497	–	–
2	[δ_0, δ_1, δ_2]	77.58	3.70838	–0.45496	(–0.01875)	–
3	[δ_0, δ_1, δ_2, δ_3]	78.84	3.73782	–0.52555	(–0.02872)	(0.02379)
4	[δ_0]	93.85	3.41092	–	–	–
5	[δ_0, δ_2]	93.98	3.46683	–	(–0.01875)	–

AICs for velocity (Experiment 3). AICs and estimated coefficients of variables were calculated for 5 models designed for running velocity in Experiment 3. The model [δ_0, δ_1] indicates that only maze had significant effects.
Single: $X = 3.19752 + r_{is} \times feeder + r_{ii}$.
Paired: $X = 3.65249 + r_{is} \times feeder + r_{ii}$.

Behavioural Brain Research 233 (2012) 577–586

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Behavioural and pharmacological effects of fluvoxamine on decision-making in food patches and the inter-temporal choices of domestic chicks

Shohei Matsunami[a,1], Yukiko Ogura[a,b,1], Hidetoshi Amita[a,b], Takeshi Izumi[c], Mitsuhiro Yoshioka[c], Toshiya Matsushima[a,*]

[a] Department of Biology, Faculty of Science, Hokkaido University, N10-W8, Kita-ku, Sapporo 060-0810, Japan
[b] JSPS fellow (Japan Society for Promotion of Sciences), Ichiban-cho 8,Chiyoda-ku, Tokyo 102-8471, Japan
[c] Department of Neuropharmacology, Graduate School of Medicine, Hokkaido University, N15-W7, Kita-ku, Sapporo 060-8638, Japan

HIGHLIGHTS

- SSRI makes chicks stay longer at a gradually depleting food patch.
- SSRI makes chicks commit less impulsive choices.
- SSRI makes chicks invest smaller efforts for food.
- SSRI makes chicks emit distress calls less frequently.
- SSRI increases 5-HT and DA levels in the medial striatum/nucleus accumbens.

ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 22 May 2012
Accepted 28 May 2012
Available online 6 June 2012

Keywords:
Serotonin
Dopamine
Optimal foraging behaviour
Impulsiveness
Effort
Medial striatum
Nucleus accumbens
Decision making

ABSTRACT

Behavioural effects of fluvoxamine (FLV, selective serotonin reuptake inhibitor) were examined in 1–2 week old domestic chicks. Chicks were tested in an I-shaped maze equipped with a feeder (*ON* feeder) that served 1 or 2 grains of millet at gradually increasing intervals, so that a depleting food patch was mimicked. By leaving the feeder, the food delivery program was reset, and chicks gained food at short intervals only after a travel to a dummy feeder (*OFF* feeder) placed on the opposite side of the maze. Chicks quickly learned to actively shuttle between the *ON* and the *OFF* feeders. FLV (intra-peritoneal injection, 20 mg/kg BW) acutely caused chicks to stay longer at the gradually depleting *ON* feeder. Inter-temporal choices were also tested, whereby two coloured beads were simultaneously presented, each associated with a small/short-delay reward or a large/long-delay alternative. FLV suppressed the choice of the short-delay option. It is suggested that an enhanced level of serotonin (5-HT) makes chicks more tolerant of the delayed food item in both behavioural paradigms. Furthermore, the decision to leave a depleting patch cannot be equated to choosing the long-delay option of the choice paradigm. Furthermore, FLV suppressed work efforts (velocity and running distance) in uncued shuttle and number of distress calls. *In vivo* microdialysis experiments revealed that FLV enhanced the extracellular concentration of 5-HT as well as dopamine (DA) locally in the medial striatum/nucleus accumbens. Underlying neuromodulatory mechanisms of behavioural control are examined in relation to locomotion, behavioural tolerance and interval timing.

1. Introduction

Because food items are unevenly distributed in nature, animals often forage by sequentially visiting one patch after another. As the food density of a patch gradually declines, the energy intake rate should inevitably decrease. Foragers should thus leave the depleting patch at an appropriate point, even though some food items are remaining. By adopting marginal value theorem, Charnov [1] argued that optimal foragers leave a patch at the point when the instantaneous gain rate matches the average gain rate available in the environment. The optimal patch-use model, as formulated by Stephens and Krebs [2], gained strong empirical support through studies of diverse animals in the wild. For example, wasps [3] and starlings [4] have been shown to behave optimally so that the long-term gain rate was maximized in the central-place resource collection.

* Corresponding author. Tel.: +81 11 706 3523; fax: +81 11 706 3523.
E-mail addresses: ysdyc223@yahoo.co.jp (S. Matsunami), y-ogura@mail.sci.hokudai.ac.jp (Y. Ogura), amita@mail.sci.hokudai.ac.jp (H. Amita), psyizumi@med.hokudai.ac.jp (T. Izumi), flute@med.hokudai.ac.jp (M. Yoshioka), matusima@sci.hokudai.ac.jp (T. Matsushima).
[1] Both authors equally contributed to the present study.

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.05.045

Some studies have tried to examine patch-use behaviours as psychological tasks performed in the laboratory. Agetsuma [5] simulated a depleting food patch by gradually decreasing reinforcements, and found that the subject monkeys stopped responding in a manner that matched the optimal patch-use model. On the other hand, Hayden et al. [6] simulated a patch-use condition and analysed neural activities of the dorsal anterior cingulate cortex. In Hayden's protocol, monkeys made choices between an option after a fixed short delay (gradually smaller drops of juice, mimicking next food in the patch) and an alternative option after a variable "travel time" (reset of the diminished return, mimicking new patch). In that study, however, the prospective "travel time" was explicitly presented, and the monkeys could make decisions in a manner similar to the inter-temporal choice paradigm. In the concurrent choices, animals generally adopt a short-sighted strategy such as maximization of instantaneous gain rate [7] or profitability [8], in contrast with the paradigm of patch-use behaviour.

The discrepancy between these two paradigms was clearly pointed out by Stephens and Anderson [9], who examined captive blue jays under two different conditions, one mimicking patch-use behaviour ("stay or leave" choices on single options), and the other inter-temporal choice (concurrent choices of "small-immediate" and "large-delayed" options). Even though the economic consequences of these two conditions were set as identical, the jays behaved differently and showed a considerable far-sightedness in the patch-use task. The authors thus argued that behavioural data obtained in the inter-temporal choice paradigm are not an "accurate guide" for understanding patch-use behaviour. However, it remains unanswered whether the neural substrates are distinct or shared between these two paradigms of behaviour.

In the present study, we examined if these paradigms could share serotonin (5-HT) as a common neuro-modulator. In inter-temporal choices tested in rats, antidepressant drugs (imipramine-like drugs and serotonin reuptake inhibitors) have been shown to acutely improve impulse control, suggesting the involvement of noradrenergic/serotonergic neuro-modulation [10,11]; null results [12,13] should also be noticed. However, 5-HT depletion by 5,7-dihydroxytryptamine infusion to raphe nuclei caused rats to commit more impulsive choices than controls [14,15], supporting the critical role of 5-HT. Functional involvement of 5-HT has also been shown by Denk et al. [16], where a blockade of 5-HT synthesis caused rats to choose an immediate reward more frequently without effects on effort-based choices. Taking these experimental findings into account, Doya [17] proposes an integrated framework in which neuromodulators (5-HT, dopamine and noradrenalin) affect economical decision making. However, the involvement of modulators has not been studied in patch-use behaviour, except for a recent genetic study in *Caenorhabditis elegans* [18].

Fluvoxamine maleate (FLV) was used as the selective serotonin reuptake inhibitor (SSRI) in this study, because FLV binds to a chicken's 5-HT transporter (SERT) at a high affinity, comparable with the human SERT [19]. If 5-HT level is increased by FLV application and if choice impulsiveness is suppressed by FLV, then FLV may also influence patch-use behaviour. FLV-treated chicks may leave a depleting food patch earlier, so that they gain a larger amount of food after a longer delay (or a new patch after a long travel time) as illustrated in Fig. 1. In addition, the effects of FLV on work efforts and distress calls were also measured.

Pharmacological effects of FLV on the striatal 5-HT level were examined by *in vivo* microdialysis. Striatal nuclei (neostriatum and nucleus accumbens [NAc] in particular) have been shown to play a critical role in inter-temporal choices, because localized lesions caused impulsive choices in rats [20] and in domestic chicks [21]. Dense 5-HT innervation of the caudate putamen by the dorsal raphe is reported in rats [22]. In chicks, similarly, NAc and the surrounding striatum are similarly innervated by 5-HT positive fibres [23].

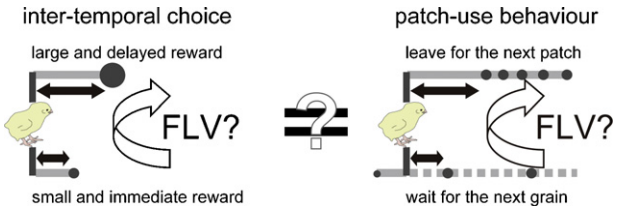


Fig. 1. Predicted effects of the fluvoxamine (FLV) on inter-temporal choice and patch-use behaviour. If the common self-control mechanism underlies the inter-temporal choice and the patch-use behaviour, the FLV-treated chicks will leave a depleting patch earlier (or, a shorter residence time; right diagram) to gain food from the next patch. This prediction comes from the assumed effect of FLV on the inter-temporal choice (left diagram), suggesting that the FLV-treated chicks will prefer a large and delayed reward to a small and immediate reward.

2. Materials and methods

2.1. Animals

A total of 193 male domestic chicks (*Gallus domesticus*, white leghorns) were used. Each chick was used once and never reused in other experiments. Thirteen chicks were discarded because they heavily emitted distress calls, therefore the present study was based on data obtained from the remaining 180 individuals; 36 chicks (experiment-1), 70 (-2), 20 (-3), 16 (-4), 18 (-5), 12 (-6), and 8 (-7), respectively. Five additional chicks served as companion individuals in the study of distress calling. New hatchlings (post-hatch day 1) were purchased from a local supplier (Hokuren Central Hatchery, Iwamizawa, Hokkaido) and were communally housed in transparent plastic cages (15 cm × 28 cm × 12 cm) that were thermo-controlled at *ca.* 30 °C under illumination by white light bulbs (12L:12D, the light period starting at 08:00). On days 2–4, chicks were fed with 1–3 g of food (per day per chick; mixture of millet and chick mash food). On day 5 and afterwards, chicks were supplied with a daily 4 g ration in addition to 0.5–1.0 g of millet gained during experiments. Water was freely available. Body weight steadily increased, maintaining *ca.* 80% of the freely fed body weight. After the end of the experiments, chicks were sacrificed by carbon dioxide if not processed for brain histology. Experiments were conducted under the guidelines and approval of the Committee of Animal Experiments of Hokkaido University. The guidelines are based on the national regulations for animal welfare in Japan (Law for the Humane Treatment and Management of Animals; after a partial amendment No. 68, 2005).

2.2. Apparatuses and drugs

2.2.1. I-shaped maze for measurement of patch residence time

An I-shaped maze was used for recording the residence time (experiment-1 and -2, Fig. 2A). The maze was composed of a pair of feeders connected by a runway (60 cm long and 13 cm wide). Each feeder was equipped with a mechanical sensor and a coloured door that was painted in blue or red. One of the feeders served as *ON* feeder that supplied grains of millet. Colour assignment was counter-balanced among individuals in each group. The sensor detected the arrival of a chick and triggered the door to open for the chick to approach the food tray. The *ON* feeder delivered food (1 or 2 grains of millet at a time) at pre-programmed intervals shown up to 25 times. Fig. 2B (a and b) illustrates the patterns of gradually diminishing food supply by plotting the cumulative gain (number of millet pellets) against the residence time (s) during which the chick stayed at the *ON* feeder. Open and filled circles denote the time at which food of 1 (a) or 2 (b) grains was delivered. For further explanation of the procedures of behavioural tasks, see Section 2.3.1.

The opposite (*OFF*) feeder did not deliver food. Visits to the *OFF* feeder terminated the food supply at the *ON* feeder, and caused the interval between grain deliveries at the *ON* feeder to be reset to the minimum value. Programmed food supply at the *ON* feeder was restarted when the chick revisited the *ON* feeder (Fig. 2C). Chicks quickly learned to forage food by shuttling between the two feeders. The I-maze was placed in a dark room, illuminated by white light bulbs, and kept at *ca.* 26–30 °C. Behaviour was monitored by a video camera placed above the maze, so that experimenters observed chicks without being seen.

2.2.2. Operant chamber for binary choice tests

An operant chamber was used for recording behaviours in the inter-temporal choice paradigm (experiment-3). A thermo-controlled box (21 cm × 19 cm × 25 cm, kept at *ca.* 27–30 °C and illuminated by light bulbs) was used [24–26]. One of the surrounding walls was equipped with a pair of holes placed side by side (separated by 3 cm, 4 cm above floor level), through which one or two coloured beads (white, blue or red) were presented for 1 s. When a chick pecked one bead, both beads were withdrawn and millet food (1 or 6 grains) was supplied to the central food tray after

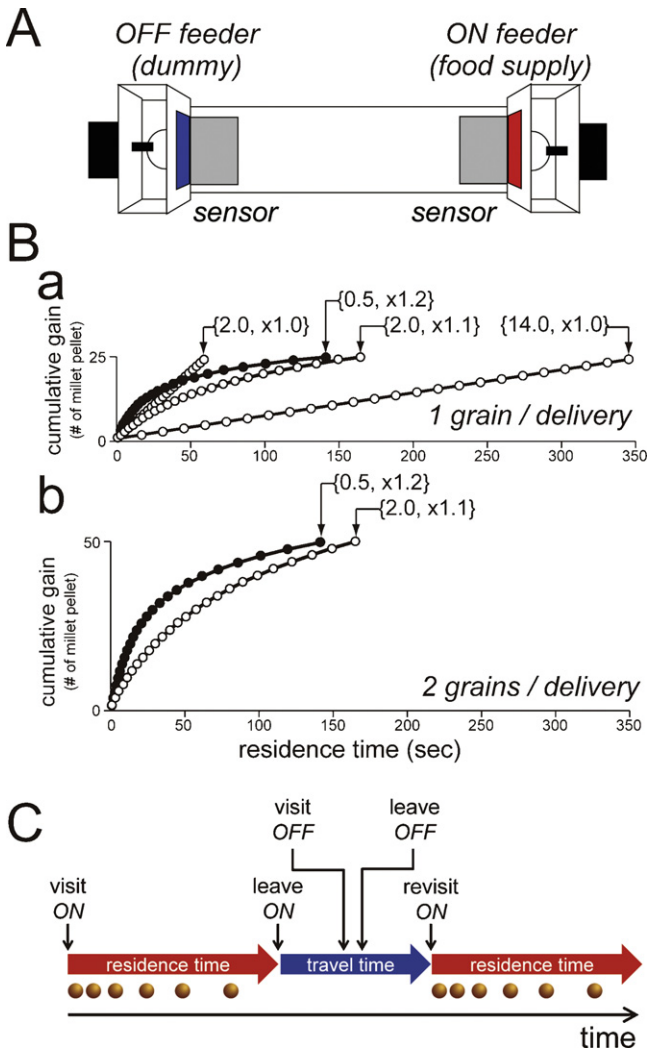


Fig. 2. Apparatus and timing of food delivery for the study of patch-use behaviours. (A) Schematic illustration of the apparatus. An I-shaped maze was composed of two feeders; *ON* feeder (red in this figure) served as a food patch, and *OFF* feeder (blue) did not supply food. As the chick arrives, the *ON* feeder starts to deliver food (1 or 2 grains of millet for each delivery) in a programmed manner as shown in (B). The *OFF* feeder similarly responds to the chick and terminates the food delivery at the *ON* feeder on the opposite side, but food is not delivered. (B) Cumulative gain (number of grains of millet) is plotted against the residence time at the *ON* feeder. Figures in parentheses $\{\alpha, \times\beta\}$ indicate the initial interval α (s), and the increment rate β. Four patterns of depletion were programmed up to a total of 25 (a) or 50 (b) grains. Two other patterns of a constant rate ($\beta = 1.0$) were also examined at short (2.0 s) or long (14.0 s) intervals. (C) Time course of food deliveries and chick's behaviour. Each ball represents delivery of a grain of millet; note the gradually increasing intervals. *ON* and *OFF* denotes the *ON* feeder and the *OFF* feeder, respectively. Leaving the *ON* feeder stopped the food deliveries, and visiting the opposite *OFF* feeder reset the interval of the *ON* feeder to the minimum value (0.5 s or 2.0 s).

a delay (0 or 1.5 s). Assignment of colours to small/short-delay food (red for 1 grain after 0 s; *SS*) and large/long-delay food (blue for 6 grains after 1.5 s; *LL*) was fixed among individuals. A white bead was always assigned to a non-rewarding option.

2.2.3. I-shaped maze for studying work efforts

An I-shaped maze (88 cm long and 12 cm wide) was used for testing each chick's foraging efforts (experiment-4); see [27] for details. Briefly, the maze was equipped with a pair of terminal feeders, each of which supplied a grain of millet at variable intervals (mean = 15 s, uniformly ranging 10–20 s) for 30 times (therefore 60 grains in total). Note the low reinforcement rate, which did not deplete during the trial that lasted for 8–9 min. The maze was not equipped with doors, and chicks freely shuttled between the two feeders and gained food if available. No visual cues were delivered but distracting motor sounds were given so that chicks were unable to associate any sensory cues with food delivery. Behaviour was recorded by a video camera placed above the maze, and trajectories of the shuttling chick were analysed off-line by using Move-tr/2D (ver 7.0) software (Library Co., Japan) according to the position of a fluorescent marker attached to the head.

Other behavioural tests (distress calls; experiment-5) and *in vivo* microdialysis (experiment-6 and -7) were conducted in the operant chamber, but chicks did not perform any specific behavioural tasks.

2.2.4. Drugs

Fluvoxamine maleate (FLV) was kindly supplied by Abbott Japan Co., Ltd. (former Solvay Pharmaceuticals B.V.)/Meiji Seika Pharma Co., Ltd. FLV was dissolved in a 0.01 M phosphate-buffered saline (pH 7.2) at concentration of 1.5 mg or 3.0 mg/ml, and intra-peritoneally administered at 1 ml/15 g body weight (BW), yielding 10 or 20 mg/kg BW, respectively. For *in vivo* microdialysis experiments (experiment-6 and -7), we used an artificial cerebrospinal fluid (aCSF) composed of: KCl 2.7 mM, NaCl 140 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.0 mM, NaH_2PO_4 0.03 mM, Na_2HPO_4 1.7 mM, pH 7.2. In the reverse microdialysis (experiment-7), FLV was added to the aCSF at concentrations of 2 or 20 μM.

2.3. Procedures of behavioural tasks

2.3.1. Patch residence time (experiment-1 and -2)

On post-hatch day 8, chicks were initially given 3 consecutive blocks of habituation to the I-maze. In each block, a communal group of 3 individuals was placed in the maze with 25 grains of scattered millet for 15 min. On days 9–11, individual chicks were tested in a block of behavioural sessions per day, and a block was composed of 20 cycles of shuttling between the *ON* and *OFF* feeders. Chicks were discarded if they stopped midway continually emitting distress calls for a period longer than 15 min.

We recorded behaviours under 6 different patterns of food supply (Fig. 2B). To avoid effects of preceding experiments, each chick was examined under one condition and not reused. In 4 patterns, the *ON* feeder delivered 1 grain of millet at a time, and up to 25 grains were supplied. In 2 other conditions, 2 grains were delivered at a time, so that the gain was 50 grains in total.

Intervals between delivery was either kept constant ($\{2.0, \times 1.0\}$ and $\{14.0, \times 1.0\}$ conditions) or gradually increased ($\{2.0, \times 1.1\}$ and $\{0.5, \times 1.2\}$ conditions). Figures in parentheses $\{\alpha, \times\beta\}$ indicate that the initial interval (between the 1st and 2nd delivery) was α s, and increment rate was β. The last interval (between the 24th and the 25th) of the depleting conditions was therefore 16 and 22 s for $\{2.0, \times 1.1\}$ and $\{0.5, \times 1.2\}$, respectively. Predictions of these experiments are schematically illustrated in Fig. 3A and B.

Four parameters (periods of time) were measured based on the sensor signals recorded by a PC using the LabView system (National Instruments Co., USA). (1) *Residence time* in the *ON* feeder: period from the arrival at the *ON* feeder until the arrival at the *OFF* feeder. (2) *Leave-point interval*: interval between the last two deliveries at the point when the chick left the *ON* feeder (Fig. 4C). (3) *Travel time*: period from the arrival at the *OFF* feeder until the arrival at the *ON* feeder. (4) *Waiting time*: period from the leave point until the point of time at which the next grain should have been delivered in the *ON* feeder (Fig. 4D).

As the first step, behaviours were recorded without any pharmacological treatments in 6 groups of chicks (experiment-1; under 4 diminishing conditions and 2 constant interval conditions, as described above). Behavioural tasks were conducted for 3 days from post-hatch day 9 to day 11. Chicks were examined in one block (20 shuttles) per day.

As the second step, the effects of FLV on *residence time* were examined in 6 other groups of chicks (experiment-2); vehicle control, 10 and 20 mg/kg BW in each of the 2 diminishing conditions. Behavioural tasks were conducted for 4 days from post-hatch day 9 to day 12. On day 9, one block (composed of 30 shuttles) was given, and chicks were pseudo-randomly allocated into 3 groups (vehicle, 10 and 20 mg/kg BW); 3 groups were thus counter-balanced based on the recorded residence time. On days 10–12, chicks received one block (composed of 20 shuttles) per day, 1 h after the FLV or vehicle solution was intra-peritoneally injected.

2.3.2. Inter-temporal choices

Effects of FLV on inter-temporal choices (experiment-3) were examined according to the procedure described previously [25]. Briefly, after initial habituation to the operant chamber on day 5, chicks were individually trained to associate colour cues with food rewards as described above. One block of single-cue training consisted of 12 pseudo-randomly arranged trials for *SS* (small/short-delay reward; red), 12 trials for *LL* (large/long-delay reward; blue), and 24 trials for no reward (white), respectively. Chicks received 2 blocks of training on day 6. On days 7–9, chicks were thereafter trained in binary choices between a rewarding bead (red or blue) and a non-rewarding white bead in one block per day. One block consisted of 12 pseudo-randomly arranged trials for *SS* (red) paired with white, 12 trials for *LL* (blue) paired with white, and 24 trials for a pair of non-rewarding white beads. The side of presentations was randomised in each trial. On day 10, the remaining chicks were allocated to two groups in a counter-balanced manner based on body weight; one group for FLV and another for vehicle treatment. At 60 min after the injection, chicks were tested in one block composed of 10 test trials of choices between *SS* (red) and *LL* (blue), which were pseudo-randomly arranged with trials for a pair of non-rewarding white beads (10 trials), a pair of *SS* (red; 10 trials) and a pair of *LL* (blue; 10 trials).

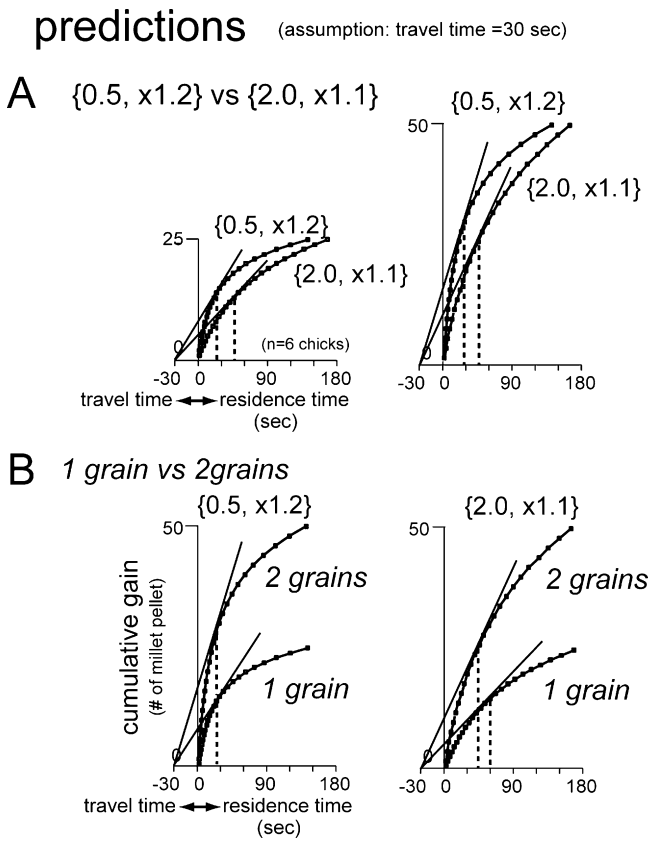


Fig. 3. Patch-use behaviour and inter-temporal choice. If chicks behave optimally, we will find that the residence time is shorter in {0.5, ×1.2} than that in {2.0, ×1.1}(A), and shorter in 2 grains than in 1 grain (B), depending on the travel time that is assumed to be 30 s in these graphs.

2.3.3. Work efforts in uncued shuttle tests

Effects of FLV on work efforts (running for feeders) were examined (experiment-4) according to the procedure described elsewhere [27]. Briefly, chicks were habituated to the I-maze on days 7 and 8, and then allocated to two groups in a counter-balanced manner based on body weight; one group for FLV and another for vehicle treatment. On days 9–11, chicks were individually tested 75 min after the FLV or vehicle solution was intra-peritoneally injected. In each test session, the two terminal feeders supplied millet grains according to the programmed food delivery that lasted for 8–9 min in total. After the food supply was terminated and all grains were consumed, chicks were left in the maze for an additional 2 min before the test session of the day was terminated.

Five parameters were measured. (1) *Total residence time* (s) in each feeder (*i.e.*, areas < 10 cm from the terminal walls). (2) *Number of visits* (times) to each feeder. (3) *Residence time per visit* (s/times) (*i.e.*, the *residence time* divided by the *number of visits*). (4) *Running distance* (cm) and (5) *velocity* (cm/s) during the 8 min period of food delivery. *Velocity* was measured in the midway after excluding the areas near (<10 cm) the feeders.

2.3.4. Distress calls

Effects of FLV on distress calls were examined at 3 doses (vehicle, 10, and 20 mg/kg BW, experiment-5). To induce distress calls, the subject chick was socially isolated from companion chicks for 3 min, as reported previously [28], and the number of distress calls was counted. Calls were also counted before and after the isolation period (the average yielded baseline level, each for 3 min), and the difference (Δ *distress calls*) was calculated. Calls were counted 20 min after the FLV or vehicle injection. FLV or the vehicle was injected every day from day 8 to day 10, and calls were measured on days 8 and 10.

2.3.5. Statistical analysis

For the data obtained in experiments 1–4, generalized linear mixed models (GLMMs) were constructed to estimate the effects of behavioural factors and the drug application by using R (ver. 2.6.0 [29]) for the platform of statistical calculations. See Tables 1–3 and supplementary tables for details. For the distress calls (experiment-5), ANOVA was used after Bartlett's test for homogeneity of variance with the significance level set at $p < 0.05$.

Table 1
Generalized liner mixed models (GLMMs) developed for the mean residence time (experiment 1).

Models	AIC	Estimated coefficients of variables		
		α_0 (intercept)	α_1 (pattern)	α_2 (grain)
1 (α_0, α_1)	72.95	3.7629	0.6190	–
2 (α_0, α_1, α_2)	73.44	3.5270	0.6182	(0.1577)
3 (α_0)	86.88	4.0725	–	–
4 (α_0, α_2)	88.12	3.8376	–	(0.1567)

AICs (Akaike Information Criteria) and estimated coefficients of variables were calculated for 4 models, which are sorted in ascending order of AIC. The chosen model (α_0, α_1) with the smallest AIC indicates that *pattern* alone was involved, whereas the number of *grain* was not. Coefficients shown in parentheses (those for α_2) indicate that the 95% confidence interval (CI) of the estimate included 0, thus the *grain* variable is not supposed significant.

Table 2
GLMMs developed for the residence time ({0.5, ×1.2} condition in experiment 2).

Models	AIC	Estimated coefficients of variables		
		β_0 (intercept)	β_1 (drug)	β_2 (day)
1 (β_0, β_1, β_2)	375.9	3.7430	0.0244	−0.1527
2 (β_0, β_2)	380.3	3.9877	–	−0.1530
3 (β_0, β_1)	390.8	3.6552	0.0264	–
4 (β_0)	394.7	3.9218	–	–

The *drug-day* model yielded the smallest AIC. The chosen model (β_0, β_1, β_2) indicates that both *drug* and *day* had significant effects.

2.4. Microdialysis

2.4.1. Surgical operation for chronic implantation of cannulae

Effects of systemic injection of FLV on centrally released serotonin (5-HT) and dopamine (DA) were examined in experiment-6 by microdialysis. To confirm its direct effects on the central nervous system, the effects of locally infused FLV through the dialysis microprobe (reverse microdialysis) was examined in experiment-7.

A guide cannula was chronically implanted on the day before the microdialysis experiment. Chicks were anaesthetized by intra-muscular injection of ketamine and xylazine (0.24 ml of a 1:1 mixture of 10 mg/ml ketamine (Sankyo Co., Japan)) and 2 mg/ml xylazine hydrochloride (Sigma, USA). Supplementary injections (0.05 ml each) were given to maintain a stable anaesthesia if necessary. Chicks were then fixed on a stereotaxic apparatus. Skin over the skull was incised, and the skull bone and dura matter were cut out to expose the brain surface. A guide cannula (AG-8, Eicom Co., Japan) was inserted into the medial striatum (mSt) of the left hemisphere. Coordinates of the probe tip were; 1.20–1.75 mm anterior from the bregma, 1.2–1.5 mm lateral from midline, and 4.3–5.3 mm from brain surface. Incised skull was covered with cotton, and the cannula was secured to the skull with dental cement. In experiment-6, a polyethylene tubing (1.0 mm o.d.) was further inserted into the peritoneal body cavity for systemic injection of FLV. The tubing was implanted under the skin and connected to a 1 ml syringe.

2.4.2. Sampling perfusate and high performance liquid chromatography (HPLC) measurement of 5-HT and DA

On the next day, an *in vivo* microdialysis experiment was conducted in a freely behaving condition without any behavioural tasks. A dialysis probe (A-I-8-02, Eicom Co.) was inserted into the brain through the guide cannula, and sampling started immediately. The active membrane of the probe (0.22 mm o.d., 2 mm long) was exposed to brain tissue. The internal space of the probe was perfused with aCSF at a rate of 1 μl/min using a syringe pump (ESP-32, Eicom Co.). Contents of 5-HT and DA in the perfusate were measured by HPLC with a reverse-phase column (PP-ODS, Eicom Co.) and an electrochemical detector (ECD-300, Eicom Co.). The mobile phase consisted of: 2.1 mM sodium 1-decansulfonate, 0.1 mM EDTA-2 Na, 0.1 M

Table 3
GLMMs developed for the residence time ({2.0, ×1.1} condition in experiment 2).

Models	AIC	Estimated coefficients of variables		
		β_0 (intercept)	β_1 (drug)	β_2 (day)
1 (β_0, β_1, β_2)	630.1	4.0951	0.0364	−0.2020
2 (β_0, β_2)	633.3	4.4799	–	−0.2017
3 (β_0, β_1)	641.9	4.0489	0.0377	–
4 (β_0)	645.0	4.4475	–	–

The *drug-day* model yielded the smallest AIC. The chosen model (β_0, β_1, β_2) indicates that both *drug* and *day* had significant effects.

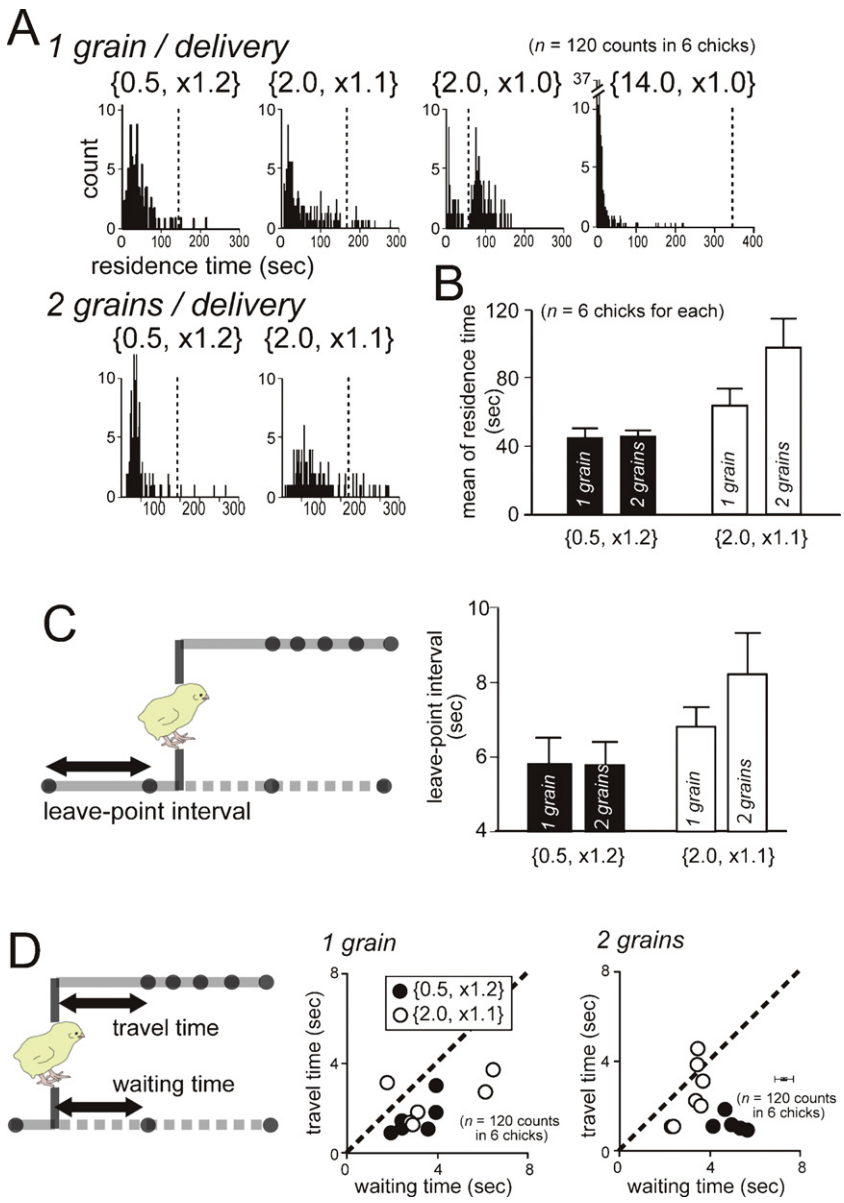


Fig. 4. Behaviours in depleting food patches (experiment-1). (A) Residence time at the food patch distributed with large variance. Histograms of residence time under different conditions are shown. Residence time was measured in 20 consecutive counts for each chick, and the total 120 counts (=20 × 6 chicks) were merged; data obtained on the last day (day 12) are shown. Vertical dashed lines indicate the terminal point at which the last grain was supplied. (B) Mean residence times of the four patterns are compared; error bars indicate S.E.M. (C) Leave-point interval. (D) Travel time (time until the next visit to the *ON* feeder) is plotted against waiting time (time to the next grain the chick could have gained if it stayed at the feeder); symbols represent individuals.

phosphate buffer and 1% (v/v) methanol (pH 6.0). A working electrode was maintained at +400 mV, and the flow pump rate was set at 0.5 ml/min. A typical example of HPLC chromatogram is shown in supplementary Fig. S1; dopamine and 5-HT were clearly separated without overlapping. Standard curve (supplementary Fig. S2) indicated that the measured signal (AUC; area under cover, in min nA) was proportionate to the concentration of dopamine and 5-HT in a range of 0.01–1 ng/ml, which covered the range of perfusate samples obtained in this study. For statistical comparisons, non-parametric tests were used at a significance level set at $p < 0.05$.

2.4.3. Histological verification

After the microdialysis experiment, the probe location was marked by injecting dye (0.1% pontamine sky blue, *ca.* 10 μl). The dialysis probe was replaced with another probe with a small hole at the tip, and the dye was injected by pressure. Chicks were anaesthetized by an injection of ketamine–xylazine cocktail (0.5 ml of a 1:1 mixture), and transcardially perfused with a fixative (4% paraformaldehyde in 0.1 mM PB, pH 7.2, 50 ml). Brains were dissected out of the surrounding tissue, post-fixed at 4 °C for 1–7 days, embedded in egg yolk, and cut into 50 μm thick frontal sections by using a vibrating microslicer (DTK-1000, Dosaka EM, Japan). Sections were mounted on aminopropylsilane (APS)-coated slide glasses, dried, stained with cresyl violet and imaged by a digital scanner.

3. Results

3.1. Behavioural effects of FLV

3.1.1. Residence time in naïve chicks (experiment-1)

The mean *residence time* was shorter in {0.5, ×1.2} than that recorded in {2.0, ×1.1} irrespective of the food amount (1 grain or 2 grains) (Fig. 4A, B). Without interval increments, chicks left the *ON* feeder after the food supply was completed (vertical dashed line, {2.0, ×1.0}) or immediately after the first or second grain ({14.0, ×1.0}). Statistical analyses were thus performed for the other 4 groups of chicks, namely {0.5, ×1.2} and {2.0, ×1.1} for 1 and 2 grains per delivery. Based on mean residence time, AICs (Akaike Information Criteria) were compared among the 4 models in which the variables of *pattern* ({0.5, ×1.2} or {2.0, ×1.1}) and food amount (1 or 2 grains) were considered (Table 1). The model with the *pattern* variable was chosen for its smallest AIC (72.95), and the model

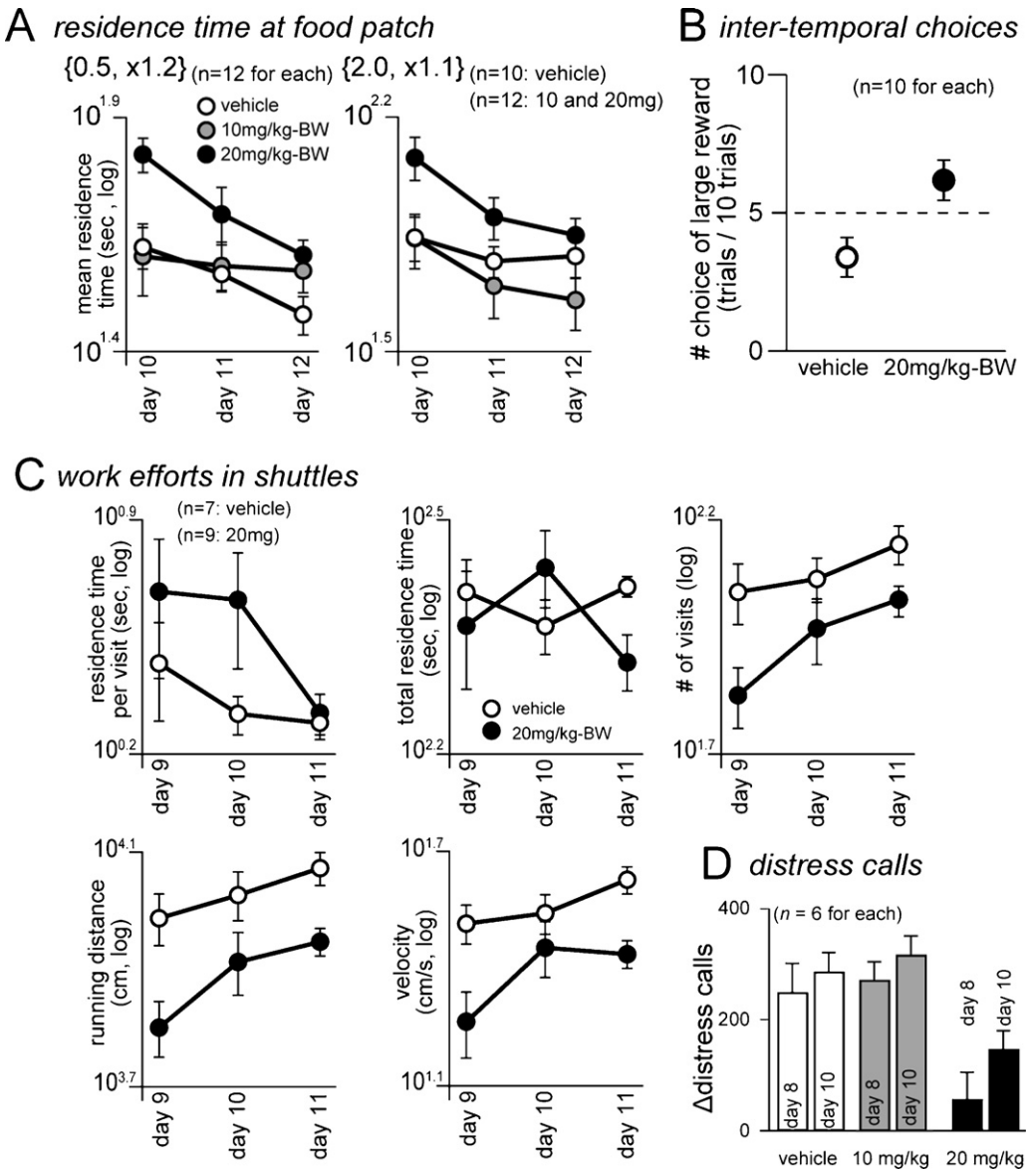


Fig. 5. Behavioural effects of FLV (fluvoxamine) on residence time (A), inter-temporal choices (B), work efforts (C), and distress calls (D). (A) Systemic injection of FLV resulted in a longer residence time. (B) FLV suppressed impulsiveness in the inter-temporal choices, but the difference was only suggestive (see text for statistics). (C) FLV decreased running distance and running velocity, but residence time per visit, total residence time and number of visits were not influenced. Means (±S.E.M.s) are plotted for the day of experiment. (D) Increase in the number of distress calls (per 3 min) elicited by social isolation.

with the second smallest AIC (73.44) contained both *pattern* and *grain* variables, although the coefficient of *grain* (as indicated in parentheses) could contain 0 value at considerably high probability ($p > 0.05$), thus the variable *grain* was not supposed to be significant. The null model gave rise to the 3rd smallest AIC (86.88), and the model with the variable grain yielded an even larger AIC (88.12), indicating that the variable *grain* should not be considered. The present results are therefore compatible with one prediction of the optimal patch-use model (Fig. 3A), that the chicks leave earlier in the higher increment rate (×1.2 rather than ×1.1). However, the results did not match with another prediction (Fig. 3B), that chicks leave earlier in the larger gain rate (2 grains rather than 1 grain). In accordance with mean *residence time* (Fig. 4B), the mean *leave-point interval* (Fig. 4C) was shorter in {0.5, ×1.2} than in {2.0, ×1.1} irrespective of the food amount.

The mean *travel time* (or the time spent in the *OFF* feeder) was also compared with the respective *waiting time* (or the time to the next delivery at the *ON* feeder) (Fig. 4D); symbols denote mean values in individuals. In both 1 grain and 2 grain trials, the *waiting time* tended to be longer than the *travel time*, even though the more profitable option (the food available by revisit after a travel time) was temporally more proximate. Chicks waited longer for the next grain particularly in the condition of {0.5, ×1.2}. Most probably, chicks behaved retrospectively based on the time after the last grain was delivered, rather than based on prospective food gains.

3.1.2. *Effects of FLV on residence time (experiment-2)*

To examine if the enhancement of endogenous 5-HT level could acutely or chronically affect patch-use behaviour, we examined FLV effects for 3 successive days. A FLV solution (or vehicle) was injected (i.p.) 1 h before the test of each day. We found that FLV elongated the mean *residence time* in both conditions acutely from the first day, but only at a high dose (20 mg/kg BW) (Fig. 5A). At a low dose (10 mg), however, significant effects were not found. We did not examine higher doses (40 mg or higher), because pilot trials revealed that higher FLV doses often caused chicks to sleep. In

the following experiments, we therefore examined the effects of 20 mg FLV. Based on residence time, AICs were compared among the 4 models in which the variables of *drug* (vehicle, 10 or 20 mg/kg BW) and *day* (10–12) were considered (Tables 2 and 3). Of these models, the full model yielded the smallest AIC (375.9 and 630.1 for the {0.5, ×1.2} and {2.0, ×1.1} conditions, respectively), and the coefficients of both variables (*drug* and *day*) included 0 value at probability $p < 0.05$, thus the *drug* effect was supposed to be significant. On the other hand, the models composed only with the *day* variables yielded a larger AIC (380.3 and 633.3, respectively), and AICs of the null models were even larger (394.7 and 645.0, respectively); significant drug effects were thus confirmed in both conditions of {0.5, ×1.2} and {2.0, ×1.1}.

3.1.3. Effects of FLV on inter-temporal choices (experiment-3)

FLV suppressed choice impulsiveness (Fig. 5B), as the number of choices of the large reward (*LL*) was higher in the 20 mg group than in the vehicle control group. Based on the number of choices of the large reward, AICs were compared between the two models in which the variable *drug* (vehicle or 20 mg) was included or not (Table S1). The *drug* model had an AIC (46.74) that was smaller than the null model (49.09), and the *drug* coefficient included 0 value at $p < 0.05$, thus the drug effect was supposed to be significant.

3.1.4. Effects of FLV on work efforts in shuttles (experiment-4)

FLV decreased running distance and running velocity, but the number of visits, total residence time and residence time per visit were not influenced (Fig. 5C). For each parameter, AICs were compared among the 4 models in which the variables *drug* (vehicle or 20 mg/kg BW) and *day* (9–11) were considered (Tables S2–S6). For *total residence time*, the null model was chosen for its smallest AIC. For *residence time per visit*, the model with the *day* variable was chosen. On the other hand, for *running distance* and *velocity*, full models were chosen with significant coefficients for the *drug* variable. For the *number of visits*, the full model was chosen. Taking all of these estimations into account, it is concluded that FLV suppressed the *total running distance* by mainly slowing down the *velocity*, but the decision to leave the uncued food patches was not influenced, in a clear contrast to FLV effects on *residence time* at the diminishing patch (experiment-2).

3.1.5. Effects of FLV on distress calls (experiment-5)

FLV suppressed the number of distress calls, suggesting a general soothing effect. Two-way ANOVAs revealed significant effects on *Δ distress calls* (Fig. 5D) of dose ($F_{dose}(2, 30) = 15.11$, $p < 0.0001$), but not of day of experiment ($F_{day}(1, 30) = 3.47$, $p > 0.05$) without interaction ($F_{dose \times day}(2, 30) = 0.27$, $p > 0.05$).

3.2. Pharmacological effects of FLV on 5-HT and DA in the striatum

3.2.1. Location of the microdialysis probes

Histological reconstruction of tracks revealed that the microdialysis probes were located in the mSt and surrounding tissues in all of the chicks studied (Fig. 6). These locations extended over the core and shell regions of the nucleus accumbens [30,31]. In some chicks, the stained track was found in the lateral part of the bed nucleus of the stria terminalis and the ventral pallidum sub-regions of the basal ganglia ventromedial to the mSt [32]. These regions in the basal ganglia are densely innervated by 5-HT positive fibres [23].

3.2.2. Microdialysis and systemic application of FLV (experiment-6)

Intra-peritoneal injection of FLV (20 mg/kg BW) elevated concentrations of both 5-HT and DA in the perfusate. Samples were collected from chicks housed in an illuminated chamber not equipped with feeders. Notice that the subject chicks did not forage food, nor did they actively walk around. Fig. 7Aa and Ac indicates the time-course of relative concentrations measured as % of the reference level (sample #0 indicated by an arrowhead). AUCs did not significantly differ between groups (Fig. 7Ab and Ad); $U = 17$, $p > 0.05$ for 5-HT; $U = 13$, $p > 0.05$ for DA (Mann–Whitney's *U*-test, $n_1 = n_2 = 6$). After the injection (samples #1–#4), on the other hand, significant differences were found; $U = 0$, $p < 0.005$ for 5-HT; $U = 0$, $p < 0.005$ for DA. It is thus suggested that FLV could have an inhibitory effect on 5-HT re-uptake, but the effect was not specific, as the DA level was also elevated. The injected FLV could indirectly increase the DA level *via* unspecified pathways, or otherwise FLV (or the elevated 5-HT) directly enhanced DA release (or re-uptake mechanisms) in the striatum. We therefore conducted a reverse dialysis experiment, in which FLV was applied to the perfusing aCSF solution.

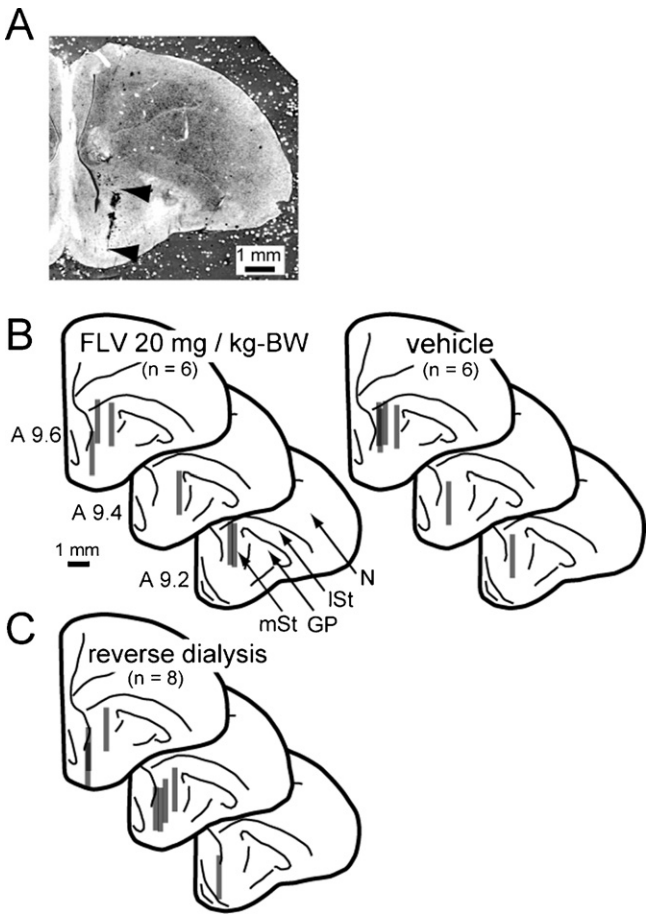


Fig. 6. Location of microdialysis probes in the striatum. (A) Representative photomicrograph of a track on a Nissl-stained section. Arrow heads point to dorsal and ventral edge of the probe stained by post-experimental injection of dye (0.1% pontamine sky blue). (B, C) Superimposed locations of probes are shown on frontal sections of telencephalon. N: nidopallium; lSt: lateral striatum; GP: globus pallidus; mSt: medial striatum.

3.2.3. Reverse microdialysis and local application of FLV (experiment-7)

Similar effects were found in the reverse microdialysis. FLV was added to the perfusate twice at 2 different doses (2 and 20 μM at samples #1 and #5, respectively), and considerable increases were found in 5-HT and DA after 20 μM (Fig. 7Ba and Bc). AUCs were compared among 3 phases of sampling, namely, samples #−4 to #−1 (aCSF), #1 to #4 (2 μM), and #5 to#8 (20 μM) by using Friedmann's

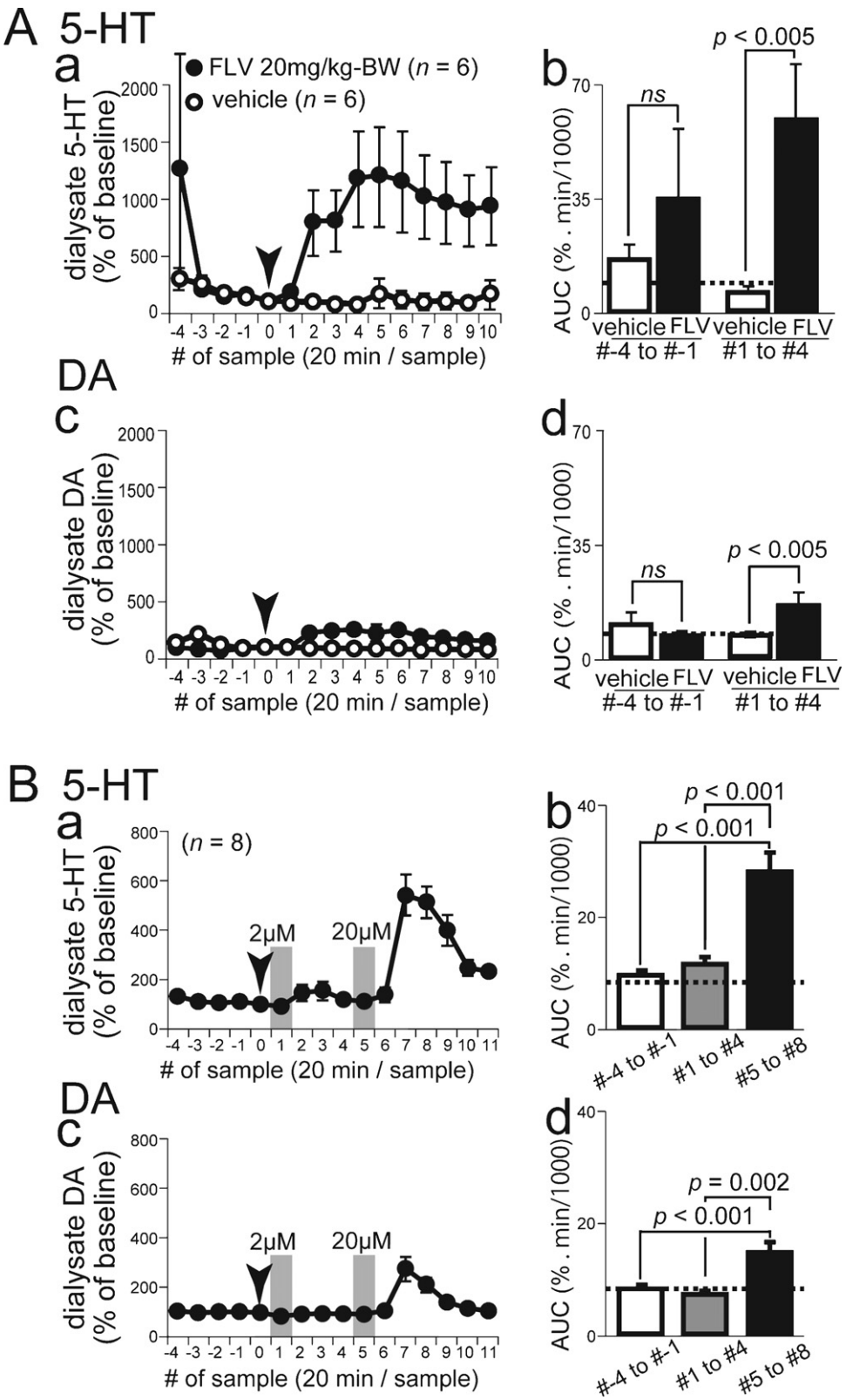


Fig. 7. Pharmacological effects of FLV on 5-HT and DA in the striatum. (A) Systemic injection of FLV (20 mg/kg BW) elevated the level of (a) 5-HT and (c) DA. Values are expressed as percent change relative to baseline (sample #0) as mean ± S.E.M. FLV (or vehicle) was intra-peritoneally injected at the beginning of the sample period #1 (arrowhead). The percent concentrations in 4 consecutive samples (namely 80 min) were summated to yield the area under curves (AUC) in b and d (mean ± S.E.M.); horizontal dashed lines indicate the baseline. AUCs were significantly different between FLV and vehicle only after the injection. (B) Local application of FLV through the probe revealed similar effects on extracellular 5-HT DA, suggesting direct action in the striatum. Time course and AUC of extracellular 5-HT and DA levels are similarly shown. AUCs were compared among three different phases of samples; samples #−4 to #−1 (aCSF), #1 to #4 (2 μM), #5 to #8 for (20 μM), and significant differences were found among these phases. Note also that the effects on DA were reproduced.

tests ($k = 3$ blocks of treatments, $m = 8$ individuals); $S = 86$ ($p < 0.01$) for 5-HT, and $S = 78$ ($p < 0.01$) for DA, respectively. We therefore conclude that FLV elevated 5-HT and DA locally in the striatum.

4. Discussion

We designed the present experiments to mimic optimal patch-use behaviour in the field. The residence time was dependent on the pattern of food supply (*pattern*) as expected, but not on the amount (*grain*) (experiment-1; Fig. 4A–C). Furthermore, the *travel time* was generally shorter than the *waiting time* at the feeder (Fig. 4D); clearly, chicks did not consider the larger gratification obtainable by leaving the patch as an alternative option. We therefore agree with Stephens and Anderson [9] that inter-temporal choice data do not give a good account for understanding patch-use behaviour.

We expected to find that chicks with a systemic FLV application would leave the depleting feeder earlier than chicks with a vehicle control (Fig. 1), under the premise that the decision to leave a food patch is identical to the choice of the large/long-delay option in the inter-temporal choices. FLV actually suppressed choice impulsiveness (experiment-3; Fig. 5B), confirming previous studies (see below for further discussion). However it is to be noticed that the effect of FLV on choices might be ascribed to altered sensitivity to delay or to amount of the food reward. We should have examined whether FLV modified the choices solely based on food amount, while the delay was identical. Further experiments should be added in future. FLV elongated residence time, contrary to our initial expectation (experiment-2; Fig. 5A). FLV also suppressed the running distance and velocity in the work effort test, as well as the distress calls induced by social isolation (experiment-4 and -5; Fig. 5C, D), suggesting a spectrum of soothing effects. *In vivo* microdialysis revealed an increase in both 5-HT and DA by systemic and local application of FLV (experiment-6 and -7; Figs. 6 and 7), suggesting the possibility that the systemic FLV directly affected the medial striatum. It is notable however that the dialysis experiments were performed without any tasks, whereas the behavioural effects of FLV were examined in chicks that were actively foraging in the maze. The increases in 5-HT and DA by FLV may appear differently under the behavioural conditions.

It is therefore concluded that (1) the present behavioural experiments only partially reproduced optimal patch-use behaviour. (2) FLV suppressed impulsiveness in the inter-temporal choices, and (3) it also made chicks tolerant of the delay in the next food gained in the patch. Finally, (4) these behavioural effects of FLV might be mediated by enhanced action of either or both of 5-HT and DA in the medial striatum.

4.1. 5-HTergic control of motor activities and residence time

FLV suppressed running distance and velocity in this study (Fig. 5C), suggesting that acutely enhanced 5-HT level decreases motor activities. In accordance with this finding, a previous report showed that a $5HT_1$ and $5HT_2$ agonist (lysergic acid diethylamide) decreased the exploratory locomotor activity of rats (see review by Geyer [33]). However, behavioural effects of 5-HT may be dependent on the context. SSRI induced hyperlocomotion in mice when tested in a novel environment, but not in a habituated environment [34]. In chicks, FLV effects should be examined in other contexts such as spontaneous motor activity in an open field without food.

On the other hand, FLV effects on residence time and the inter-temporal choices (Fig. 5A and B) may be explained differently, whereby 5-HT enhances the tolerance for delay of the forthcoming reward. The present findings are in concert with previous studies of behavioural pharmacology ([10,11] for acute effects of 5-HT uptake inhibitor, [11,14,15] for 5,7-DHT infusion to dorsal raphe) and a recent neurophysiological analysis of raphe neurons ([35]; also see [36] for a microdialysis study in the dorsal raphe nucleus). It must however be noticed that the acute effects of SSRIs have not been reproduced in inter-temporal choice tests using rats, in which subjects made choices by lever-pressing [12,13]. In these studies, the discrepancy with Bizot et al. [10] has been ascribed to different task conditions.

Acute effects of SSRIs have also been questioned in a study using pigeons [37]. They adopted behavioural titration for inter-temporal choices and examined a series of SSRIs (fluoxetine, citalopram and paroxetine; systemic application), but found only chronic effects that appeared after consecutive application for 17 days. The present results, on the other hand, revealed acute effects of FLV in chicks (Fig. 5B). The discrepancy may be ascribed to the different affinities of these SSRIs to the avian SERT [19]. While FLV strongly inhibits the chicken SERT (1.2 times in terms of K_i compared to human SERT), the SSRIs used [37] had lower levels of inhibition (fluoxetine 5.6, citalopram 41 and paroxetine 41, respectively).

4.2. 5-HT and interval timing (or retrospective/immediate sense of time)

Involvement of DA in the control of the internal clock has gained substantial experimental support (see reviews by Meck [38,39]). Haloperidol, a D_2 receptor antagonist, is reported to delay the internal clock [40]. It is assumed that the corticostriatal circuit responsible for the internal clock is under speed-control by DA actions from the substantia nigra. However, significant action of 5-HT on the timing mechanism is questioned. Ho et al. [41] review recent studies of 5-HT actions on interval timing. It is notable that 5-HT depletion by 5,7-DHT application increases the Weber fraction of the timing behaviour. However, they argue that 5-HT does not change the speed of the internal clock, but is involved in behavioural switching. Ho et al. [42] actually report that systemic FLV does not influence indices of behavioural timing tested by a fixed-interval peak procedure.

It might be argued that the FLV effect on residence time (Fig. 5A) is explained by its action on interval timing, particularly through the unexpected increase in DA level by FLV (Fig. 7). However, the enhanced DA level should shorten the residence time by overestimation of the perception of time, if its action is similar to amphetamine [39]. It is thus not plausible that the observed effects are due to the accompanying DA increase. In conclusion, we may suppose that the perception of interval timing was not influenced by FLV in the present study.

4.3. DA release through 5-HT enhanced by FLV

Systemic application of FLV increased not only the 5-HT but also the DA level in the striatum (Fig. 7A), which was reproduced in the reverse dialysis experiment (Fig. 7B), indicating that FLV increased DA locally in the striatum. As reviewed by Navailles and De Deurwaerdère [43], it is possible that the enhanced 5-HT could directly act on the dopaminergic terminals, making the DA transporter (DAT) reverse the direction of dopamine release. A similar mechanism may underlie the DA increase by FLV found in the present study. Alternatively, it is possible that FLV directly acted on the DAT and inhibited the DA uptake. Although data are not available on the avian DAT, a biochemical study has revealed that binding of FLV to human DAT is negligibly small [44]. The cellular mechanisms underlying the DA release by FLV need further investigation.

Co-release of 5-HT and DA by a 5-HT reuptake blocker (alaproclate) has already been reported in an *in vivo* experiment [45] and also in *in vitro* preparations of striatum using an SSRI (fluoxetine, [46]), but the functional significance remains unknown. Further

detailed experiments are needed to reveal the functional significance of the cross talks between 5-HT and DA.

5. Conclusions

Systemic application of FLV caused chicks to stay longer at a gradually depleting food patch. FLV also suppressed impulsiveness in inter-temporal choices, suggesting that an enhanced 5-HT level made chicks more tolerant of the delayed food reward in both behavioural paradigms. On the other hand, the decision to leave a depleting patch cannot be equated to the choice of the large/long delay option of the inter-temporal choice paradigm. The microdialysis study revealed that FLV enhanced the extracellular concentration of 5-HT as well as DA in the medial striatum/nucleus accumbens. In addition to 5-HT, DA in the striatum may be involved in tolerance control.

Acknowledgements

This study was supported by a grant-in-aid for scientific research from the Japan Society for the Promotion of Science (#22570070) and from the Japanese Ministry for Education, Culture, Sports, Science and Technology (#22120502) for Innovative Area on the "Study of the neural dynamics for understanding communication in terms of complex hetero systems."

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2012.05.045.

References

[1] Charnov E. Optimal foraging, the marginal value theorem. Theoretical Population Biology 1976;9:129–36.
[2] Stephens DW, Krebs JR. Foraging theory. New Jersey: Princeton University Press; 1986.
[3] Kasuya E. Central place water collection in a Japanese paper wasp, *Polistes chinensis antennalis*. Animal Behaviour 1982;30:1010–4.
[4] Kacelnik A. Central place foraging in starlings (*Sturnus vulgaris*). I. Patch residence time. The Journal of Animal Ecology 1984;53:283–99.
[5] Agetsuma N. Simulation of patch use by monkeys using operant conditioning. Journal of Ethology 1998;16:49–55.
[6] Hayden B, Pearson J, Platt M. Neuronal basis of sequential foraging decisions in a patchy environment. Nature Neuroscience 2011;14:933–9.
[7] Mazur JE. Optimization theory fails to predict performance of pigeons in a two-response situation. Science 1981;214:823–5.
[8] Matsushima T, Kawamori A, Bem-Sojka T. Neuro-economics in chicks: foraging choices based on amount, delay and cost. Brain Research Bulletin 2008;76:245–52.
[9] Stephens DW, Anderson D. The adaptive value of preference for immediacy: when shortsighted rules have farsighted consequences. Behavioral Ecology 2001;12:330–9.
[10] Bizot J, Thiebot M, Le Bihan C, Soubrie P, Simon P. Effects of imipramine-like drugs and serotonin uptake blockers on delay of reward in rats. Possible implication in the behavioral mechanism of action of antidepressants. Journal of Pharmacology and Experimental Therapeutics 1988;246:1144–51.
[11] Bizot JC, Le Bihan C, Puech AJ, Hamon M, Thiébot MH. Serotonin and tolerance to delay of reward in rats. Psychopharmacology 1999;146:400–12.
[12] Charrier D, Thiébot M. Effects of psychotropic drugs on rat responding in an operant paradigm involving choice between delayed reinforcers. Pharmacology Biochemistry and Behavior 1996;54:149–57.
[13] Evenden J, Ryan C. The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology 1996;128:161–70.
[14] Wogar MA, Bradshaw C, Szabadi E. Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. Psychopharmacology 1993;111:239–43.
[15] Mobini S, Chiang TJ, Ho MY, Bradshaw CM, Szabadi E. Effects of central 5-hydroxytryptamine depletion on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology (Berlin) 2000;152:390–7.
[16] Denk F, Walton M, Jennings K, Sharp T, Rushworth M, Bannerman D. Differential involvement of serotonin and dopamine systems in cost–benefit decisions about delay or effort. Psychopharmacology 2005;179:587–96.
[17] Doya K. Modulators of decision making. Nature Neuroscience 2008;11:410–6.
[18] Bendesky A, Tsunozaki M, Rockman M, Kruglyak L, Bargmann C. Catecholamine receptor polymorphisms affect decision-making in *C. elegans*. Nature 2011;472:313–8.
[19] Larsen M, Elfving B, Wiborg O. The chicken serotonin transporter discriminates between serotonin-selective reuptake inhibitors. A species-scanning mutagenesis study. Journal of Biological Chemistry 2004;279:42147–56.
[20] Cardinal R, Pennicott D, Sugathapala C, Robbins T, Everitt B. Impulsive choice induced in rats by lesions of the nucleus accumbens core. Science 2001;292:2499–501.
[21] Izawa E-I, Zachar G, Yanagihara S, Matsushima T. Localized lesion of caudal part of lobus parolfactorius caused impulsive choice in the domestic chick: evolutionarily conserved function of ventral striatum. Journal of Neuroscience 2003;23:1894–902.
[22] Waselus M, Galvez J, Valentino R, Van Bockstaele E. Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum. Journal of Chemical Neuroanatomy 2006;31:233–42.
[23] Metzger M, Toledo C, Braun K. Serotonergic innervation of the telencephalon in the domestic chick. Brain Research Bulletin 2002;57:547–51.
[24] Aoki N, Csillag A, Matsushima T. Localized lesions of arcopallium intermedium of the lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary choice task. European Journal of Neuroscience 2006;24:2314–26.
[25] Amita H, Kawamori A, Matsushima T. Social influences of competition on impulsive choices in domestic chicks. Biology Letters 2010;6:183–6.
[26] Amita H, Matsushima T. Instantaneous and cumulative influences of competition on impulsive choices in domestic chicks. Frontiers in Neuroscience 2011;5:101.
[27] Ogura Y, Matsushima T. Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks. Frontiers in Neuroscience 2011;5:91.
[28] Hayashi I, Ono Y, Matsushima T. Visual cues for suppressing the isolation-induced distress calls in newly hatched quail chicks. Zoological Science 2001;18:1065–71.
[29] R Development Core Team. R version 2.12.0.: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2010, http://www.r-project.org.
[30] Bálint E, Csillag A. Nucleus accumbens subregions: hodological and immunohistochemical study in the domestic chick (*Gallus domesticus*). Cell and Tissue Research 2007;327:221–30.
[31] Bálint E, Mezey S, Csillag A. Efferent connections of nucleus accumbens subdivisions of the domestic chicken (*Gallus domesticus*): an anterograde pathway tracing study. Journal of Comparative Neurology 2011;519:2922–53.
[32] Reiner A, Perkel DJ, Bruce LL, Butler AB, Csillag A, Kuenzel W, et al. Revised nomenclature for avian telencephalon and some related brainstem nuclei. Journal of Comparative Neurology 2004;473:377–414.
[33] Geyer MA. Serotonergic functions in arousal and motor activity. Behavioural Brain Research 1995;73:31–5.
[34] Brocco M, Dekeyne A, Veiga S, Girardon S, Millan MJ. Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake. A pharmacological characterization of diverse classes of antidepressant agents. Pharmacology Biochemistry and Behavior 2002;71:667–80.
[35] Miyazaki K, Miyazaki KW, Doya K. Activation of dorsal raphe serotonin neurons underlies waiting for delayed rewards. Journal of Neuroscience 2011;31:469–79.
[36] Miyazaki KW, Miyazaki K, Doya K. Activation of central serotonergic system during work for delayed rewards. European Journal of Neuroscience 2011;33:153–60.
[37] Wolff M, Leander J. Selective serotonin reuptake inhibitors decrease impulsive behavior as measured by an adjusting delay procedure in the pigeon. Neuropsychopharmacology 2002;27:421–9.
[38] Meck WH, Benson AM. Dissecting the brain's internal clock: how frontal–striatal circuitry keeps time and shifts attention. Brain and Cognition 2002;48:195–211.
[39] Coull JT, Cheng RK, Meck WH. Neuroanatomical and neurochemical substrates of timing. Neuropsychopharmacology 2011;36:3–25.
[40] MacDonald CJ, Meck WH. Differential effects of clozapine and haloperidol on interval timing in the supraseconds range. Psychopharmacology 2005;182:232–44.
[41] Ho MY, Velázquez-Martínez D, Bradshaw C, Szabadi E. 5-Hydroxytryptamine and interval timing behaviour. Pharmacology Biochemistry and Behavior 2002;71:773–85.
[42] Ho MY, Al-Zahrani SSA, Velazquez Martinez D, Lopez Cabrera M, Bradshaw C, Szabadi E. Effects of desipramine and fluvoxamine on timing behaviour investigated with the fixed-interval peak procedure and the interval bisection task. Psychopharmacology 1996;125:274–84.
[43] Navailles S, De Deurwaerdère P. Presynaptic control of serotonin on striatal dopamine function. Psychopharmacology (Berlin) 2011;213:213–42.
[44] Owens MJ, Knight DL, Nemeroff CB. Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. Biological Psychiatry 2001;50:345–50.
[45] Yadid G, Pacak K, Kopin IJ, Goldstein DS. Endogenous serotonin stimulates striatal dopamine release in conscious rats. Journal of Pharmacology and Experimental Therapeutics 1994;270:1158–65.
[46] Zhou FM, Liang Y, Salas R, Zhang L, De Biasi M, Dani J. Corelease of dopamine and serotonin from striatal dopamine terminals. Neuron 2005;46:65–74.

LIST OF OTHER PUBLICATIONS

1. 松島俊也，Chentao Wen，小倉有紀子，網田英敏：
「競争・同調・社会的促進」計測自動制御学会誌（*計測と制御*），
52 巻，3 号，pp. 189-194（2013）

競争・同調・社会的促進

松島俊也*・Chentao WEN**・小倉有紀子**
網田英敏**

*北海道大学 大学院理学研究院　北海道札幌市北区北 10 条西 8
**北海道大学 大学院生命科学院　北海道札幌市北区北 10 条西 8
*Graduate School of Science, Hokkaido University, N10 W8, Kita-ku, Sapporo, Hokkaido, Japan
**Graduate School of Life Science, Hokkaido University, N10 W8, Kita-ku, Sapporo, Hokkaido, Japan
*E-mail: matusima@sci.hokudai.ac.jp

キーワード：最適採餌理論 (optimal foraging theory)，異時点間選択 (inter-temporal choice)，価値関数 (value function)，社会的採餌 (social foraging)，選択衝動性 (choice impulsiveness).
JL 0003/13/5203–0189

1. 概要

神経経済学はしばしば，古典的な最適採餌理論に依拠してきた．これは 1 頭の採餌者だけを考え，「その利益を最大にする決定（最適な決定）は何か」を問うものであった．しかし，なんらかのシナジー効果があれば群れが形作られる．さらに配分可能な餌なら，群れの中の各個体は producer（生産者）と scrounger（掠奪者）という 2 つの戦術に分化する．ここで単純な分配則を仮定すると，採餌決定について以下のような予想が生まれる．社会採餌は資源競争を介して（1）選択衝動性を亢進し，（2）エフォート投資を亢進するとともに，（3）個体の運動を同調させる．その際，長期的な見通しや予測，群れ全体を統括する情報センターなどを仮定する必要はないし，個体は最適性を実現できない．局所的な相互作用があるならば，各採餌者がその自己の利益の逐次改善を近視眼的に追及する限り，群れには様々な行動特性の共有と同調が創成する．この総説では，広くみられる社会採餌について，その微視的なプロセスを整理したい．集団の巨視的な挙動を実現するにあたって，個体がいかなる行動特性を備えているか，これを検討する．さらにヒヨコを対象とした単純な実験（行動と神経科学）によって得られた知見を紹介し，社会採餌行動を特徴づける要因を具体的に示したい．

2. 準備：採餌行動から

2.1 最適者の生存

「最適者の生存」(survival of the fittest)，この言葉は 19 世紀半ば，チャールズ・ダーウィンによって提案された進化現象の説明（進化論）を一言で言い表わす用語として，知られている．動物の形質（体のつくりや行動の仕方）には遺伝性を伴うばらつきが個体ごとにあって，これを変異と呼ぶ．特定の変異形質を持つ個体が，それを持たない他の個体に比べてわずかに有利であって，より多くの子（あるいは 2 世代後の孫）を生み出すことがあるとしよう．この最適者（fittest）の形質はやがて，たくさんの世代を交代する長い時間の果てにあっては，2 位以下の他の形質をすべて排除する優先形質になるだろう，と考えるものである．後者の過程を選択と呼ぶ．変異と選択，この 2 つを鍵として成り立つ過程を「最適者の生存」と呼んでいるのである．

この概念は，実に多くの誤解を招いてきた．多くの辞書では（また通常の理解では）「最」の文字を取り去って，「適者生存」と理解する．さらには「弱肉強食」という，単に食物連鎖しか意味しない言葉とも，同一と見なされる場合すらある．まことに生物学の用語は混乱（誤用）を招きやすい．あるいは意図をもって利用（悪用）されやすい．

生物学の用語が人間の社会に適用されるときには，しばしば十分な疑いを持ってかかるべきだ．「最適者の生存」もその一例である．個人の利得を貪欲に最大化する行為を無条件に是認し，それを合理化する文言として使われる場合が多いからである．価値に関わる議論（νομος）が，検証可能な科学（λογος）の顔をして語られる場合も多い．実際，何が最大化されるべきなのか．まぎれなく計測できる変数として一意に書き下せるのだろうか．計るべき値を正しく計測できているだろうか．自明な話ではないのである．

実際，われわれが生きる世界は，はるかに多様なもので満ちている．植物や動物の図鑑の 1 ページを見るだけで十分だろう．多種多様な生物が世界を満たしている．唯一の最適者が無数にコピーをふやす，そのような枠組みは明らかにわれわれの直観に反するのである．ならば，「最適者の生存」，つまり変異と選択はどのように働いているのか．「最適性」は機能しているのか．この記事では，群れの採餌経済という現象を軸に，この問いを考えてみたい．その前に，1 頭の動物だけ存在する世界を考えよう．

2.2 最適採餌理論

餌を採る時，良い餌もあれば，悪い餌もある．「良い」とは何か．

良さを計る単位を「通貨」と呼ぶ[1] が，ここでは 1 個体が単位時間当たりに上げる利益（エネルギー利得），いわゆる利益率を「通貨」とする．利益率は時間とともに変動するが，エネルギー保持量（備蓄）がある程度は期待できるので，長期的に平均した値[2] を考えるのが妥当である．変

動に対する行動特性については，リスク感受性という別の要因を考える必要がある．リスクの議論は別に譲って[3]，本稿では割愛する．

では，長期平均利益率を最大にするために，動物は長期的な見通しや予測を持つ必要があるのだろうか．もし，すべての餌資源の空間分布が未来にわたって見える，そのような世界に生きていれば，そうだろう．しかしこれは現実的ではない．動物は限られた範囲しか見ないし，餌との遭遇も偶発的である．E. Charnov は 1976 年，この拘束条件のもとで利益率を最大にする解を求めた[4]（**図 1**）．

世界に n 種類の餌があると仮定する．タイプ $i(=1\ldots n)$ の餌は，エネルギー利得 (e_i) を与える．ある餌は大きく，ある餌は小さい．また，処理に要する時間（つまり，手に入れてから消費し尽くすまでの時間的な投資）についても変異があり，処理時間 (h_i) を要すると仮定する．ある餌は手間がかからず，ある餌は手間がかかる．この比 (e_i/h_i) を餌の利潤率（profitability）と呼ぶ．

長期平均利益率 R は，餌との遭遇率を λ_i，その餌を採る確率を p_i として，つぎの式で与えられる．

$$R = \frac{\Sigma p_i \lambda_i e_i}{1 + \Sigma p_i \lambda_i h_i} \qquad (1)$$

ある餌に出会ったとき，これを啄ばむ（peck する）べきか，すべきではないのか，これが問題である．これは，$\{e_i, h_i, \lambda_i\}$ を既知数としたとき，R を最大にする $\{p_i\}$ の組を求めよという，単純な解析の問題に帰着する．

啄ばまなければ，この処理時間を探索に割り振ることができる．それによって，動物は別のより良い餌に出会うかもしれない．（もちろん，出会わないかもしれない．）その期待値を喪失利益と呼ぶ．もちろん，動物は演繹的にこれを解くことはせず，得ていた餌の経験から喪失機会を推定するわけである．

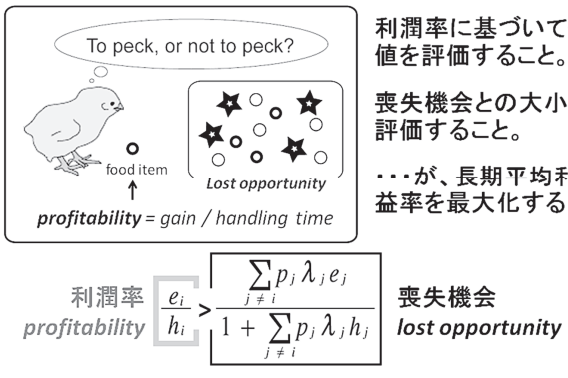


図 1　最適採餌理論

ある餌（**food item**）に出会ったとき，それを採るべきか否か，これが問題である．**peck** すれば（啄ばめば）利益 (e_i) を得る．しなければ，処理時間 (h_i) を探索に回すことで，新しい餌に遭遇する機会を得る．どちらが良いか，というということだ．採ることの利益とは「利潤率」，採らないことの利益は「喪失機会」である．価値評価さえ正確ならば，「よりましな」選択肢を絶えず選び続けることで，採餌者は長期利益率を最大化することができる．長期的な見通しを持つ必要はない．

問題は利潤率と喪失機会の大小関係だと Charnov は主張したのである．動物が持つ知識も予測も限定的である．だから，その中で最適な行動とは，第 1 には，目の前の確実な餌について，利潤率を正確に推定することである．近視眼的で良い．第 2 には，喪失機会を閾値として，より良い方を選ぶことである．これは選択の問題である．

Charnov の理論[4] はその後，E.W. Stephens と J.R. Krebs[1] によって整理された．さらに実証的な研究の結果，多くの動物が利潤率に基づく選択を行っていることが判明した．

2.3　異時点間選択

類似の問題が人の選択行動を扱う心理学の世界から独立に提案されていた．異時点間選択[5] と呼ばれる課題である．報酬に遅れがあれば価値を割引く，という論理である．これは人間の金融の原則でもある．今確保できない利益は，待たねばならぬ時間に応じて，その価値を減じて考えるべきである．貸した金は利息を付けて返してもらうべきだし，手形の取引価格は額面から割引かねばならない（**図 2**）．

ここで問題となるのは，割引きを与える関数である．金融では単位時間あたり一定の割引率を考えるから，これは時間に対して指数的に割引を行うことを意味する．すなわち価値 V を遅延時間 t の関数として

$$V = e^{-\gamma t} \qquad (2)$$

で与えるものである．強化学習[6] でも同じ式が受け入れられている．

これに対して，Ainslie は Herrnstein[7] による選択行動の研究を受け，時間的割引きは双曲線関数で与えることが妥当である，と主張した．すなわち

$$V = \frac{1}{1 + kt} \qquad (3)$$

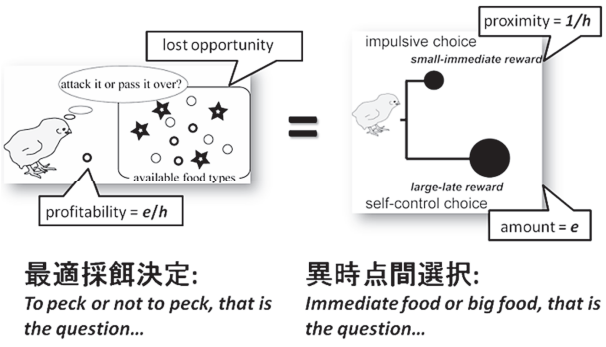


図 2　最適採餌と異時点間選択

「採るべきか，採らざるべきか，それが問題だ …」これが最適採餌問題における決定である．「近い餌を採るべきか，大きな餌を採るべきか，それが問題だ …」これは異時点間選択における決定である．**2** つの問には，見かけ上の類似性がある．量という価値と，近さという価値と，両者の間に拮抗（コンフリクト）があるときの決定を問題にしているからである．

がより良く人間の選好を説明する，と主張したのである．

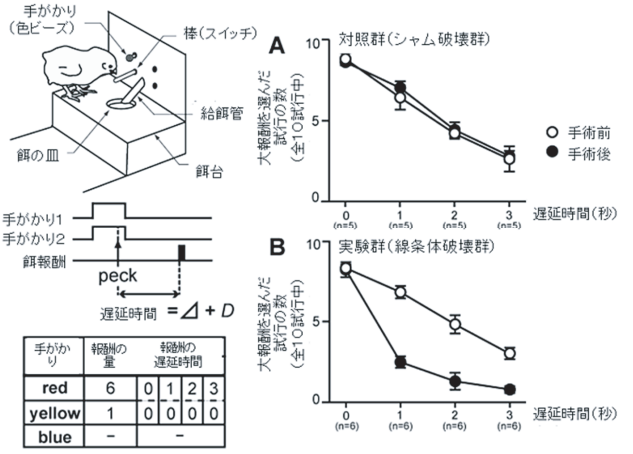
われわれは双曲線関数による割引き（(1) 式）が，最適採餌理論における利益率（(3) 式）とも似ていることに着目した．最適採餌理論と異時点間選択は，もちろん同一ではない．しかし，前者が利潤率 (e/h) の正確な推定に依存するに対して，後者は近さ $(1/h)$ と量 (e) の間の選択に関わる．限定的だが，両者は共通性を持つのである．

実際，ヒヨコを用いた行動実験の結果，利潤率は異時点間選択の結果を良く説明することがわかった．

2.4 ヒヨコの選択

簡単に実験の手順を説明しよう[8]．色のついたビーズを見せると，孵化後まもないヒヨコは活発に啄ばむ．その時，数秒の遅れで，1 粒か 6 粒の粟を与える．このような経験を 1 日 30 分程度，数日にわたって繰り返すと，ヒヨコは速やかに色と餌との強固な連合を形成する．赤ならば，6 粒の餌が 0–3 秒の遅延（個体ごとに異なる遅延とする）で与えられる．黄なら 1 粒がすぐに（0 秒の遅延で）与えられる．青なら餌はない（**図 3**）．

ヒヨコが餌の量 (e) と近さ $(1/h)$ を予期していると考える根拠が，この実験から得られた．テストでは 2 つのオプションを同時提示し，最初の 10 試行の内で何回，赤を選び 6 粒を獲得したか，これを縦軸に示したものである．横軸に赤の（6 粒の）遅延時間をとった．図の○で示したデータに注目いただきたい．0 秒なら赤をより多く選ぶ．3 秒なら黄を選ぶ．このことは，量と遅延によって餌を評価していることを示唆する．



手がかり	報酬の量	報酬の遅延時間			
red	6	0	1	2	3
yellow	1	0	0	0	0
blue	–	–			

図 3　異時点間選択と線条体（側坐核）

ヒヨコは視覚的手がかり（ビーズ，**LED** の色）と餌報酬（粟の量と遅延時間）を速やかに連合する．トレーニング試行では，選択肢をひとつだけ与える．テスト試行では **2** つの選択肢（**2** つの色，赤と黄）を同時に提示し，ヒヨコがどちらを啄ばんだか，を記録する．**10** 回の試行の中で赤を啄ばんだ回数を記録し，平均値（± 標準誤差）を示したものが右の上下のグラフ（○）である．遅延が **0** 秒で学習させたヒヨコは，平均 **8** 回まで赤を選んだ．**3** 秒の群では，約 **3** 回となった．**3** 秒という時間が，きわめて強い時間割引きをもたらしたことが判る．線条体（側坐核）を実験的に破壊した場合（下段 **B** の●），わずかな遅延でも嫌うようになった．

これは，奇妙な話である．二者の良し悪しを正確に評価しているなら，ヒヨコは 100%，より良いオプションだけを選び取っても，良さそうなものだ．二者の間にわずかでも差があれば，より良い選択だけを常に選び取ることが最適な行動である．しかし，現実のヒヨコは対応則（matching law，マッチングの法則とも言う）によっている．対応則は心理学者 Herrnstein によって提唱された経験則で，強化子（報酬など）の「良さ」の程度に応じて，選択を割り振る，とするものである．状況に応じて逐次改善を図るには適切な方略であるが，利益を最大化するものではない．実際，従来の動物の野外観察の結果と一致する．ヒヨコも例にもれず，最適性から逸脱した（利益の最大化を実現できない）選択を行っているのである[9]．

以上，進化・動物行動・心理をまたいで，採餌を理解するための基礎を論じた．いずれも，世界に 1 個体だけを置いて考えている．次章では，群れのなかで採餌する状況を見よう．群れは個体の採餌決定にどう介入するだろうか．

3．群れの採餌経済

3.1 群れのサイズを決めるもの

そもそも，なぜ群れるのか？シャチの行動の研究 Baird & Dill (1996)[10] を見てみよう．回遊型のシャチは母系の血縁（母と娘たち）を単位とする群れ（pod）を単位として行動する．1 つの pod は小さく，1–3 頭からなる．実際，1 頭で採餌する（アザラシを狩る）より 2–3 頭の方が狩りは効率的で，1 頭当たりの収益は 3 頭の時に最大となる．3 頭が最適な採餌を実現するのである．しかし，しばしばいくつかの pod が一時的に 1 つの採餌集団を形成して，群れの個体数は最大 15 頭に及ぶこともある．個体あたりの収益を下げてまで，集団を大きくなる理由は何だろうか（**図 4**）．

Giraldeau & Caraco (2000)[11] はこの現象を取り上げた．規範や血縁など，社会的な統制が群れへの加入に影響しない場合，3 頭という最適な状態は，安定な均衡[(注1)]にはなりえない，と彼らは指摘する．10 個体より小さな群れであれば，無条件に加入すべきである．私が加入することによって 1 頭当たりの利得が減るとしても，私が単独で採餌する場合に比べれば，より大きな利得がある．このように，群れへの加入は，シナジー効果を相殺するところまで進む．

3.2 生産者・掠奪者ゲーム

個体が柔軟に行動を切り替えることが可能ならば，2 つの戦術が分化しやすい．1 つの戦術は，餌との遭遇を求めて動くことである．これを生産者（producer）戦術と呼ぶ．もう 1 つは，他の生産者が餌を発見したら，近寄って餌を掠奪することである．掠奪者（scrounger）戦術と呼ぶ．

ここにも，頻度依存性があることに注意してほしい．群れのすべての個体が生産者戦術を取る時，この群れに紛れ

(注1) 平衡解ともいうが，ここではゲーム理論での訳語に従う．

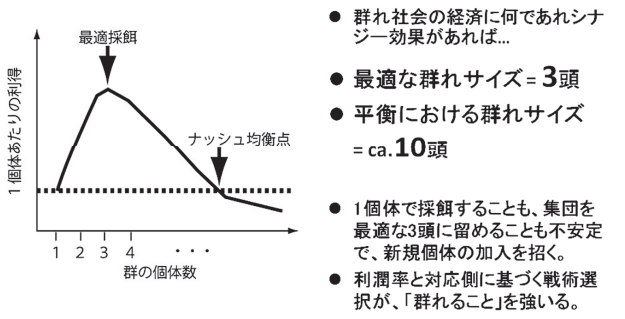


図 4　群れサイズの安定均衡解

シャチはアザラシなどを狩るとき，複数の血縁小群（pod）が集まって大きな群れとなる．3 頭が最適であって個体の利益は最大となるが，現実の群れは 10 頭を超えて均衡に至る．この均衡点では群れに加わるシナジー効果は相殺されてしまう．

込んだ 1 頭の掠奪者は大きな利益を上げることができる．当然，利潤率の逐次改善を求めて，掠奪者戦術へと切り替わる個体が生まれる．Giraldeau はムクドリの群れの行動を調べ，同一個体が速やかに 2 つの戦術を切り替えると報告している．両者は視線の方位が異なるという．6–7 割の鳥は生産者として眼を地面に向け，残りの掠奪者は水平面に向けて生産者を見ているのだという．

利益の逐次改善に向けて速やかに戦術を切り替えていく限り，戦術の割合（頻度）は均衡に達する．この時，「採餌時間の何割を生産者として働き，残りの何割を掠奪者として振る舞うか」が，公共情報として群れに共有されることになる．しかし，ここで「情報センター」のような装置（社会的制度）は必要ではない．長期にわたる見通しと予測も必要とはしない．個体間の相互作用が，近視眼的な逐次改善へ向かう個体の群れに，秩序を創成するのである．

この考えは，Noe らによる生物市場[12] と基本的に同じである．明確な例が，霊長類の baby market の現象に見いだされている．他のメスが産んだ子であろうと，サル達はは生まれたばかりの赤ん坊を抱くことを好む．そのためには赤ん坊の母親に毛づくろい（グルーミング）を行い，これが対価としなければならない．その群れに赤ん坊が少ない時ほど，グルーミング時間は長くなることが報告されている[13]．赤ん坊の対価がグルーミング時間を通貨として，この市場を構成する個体間で共有されているのである．

さて，群れの中で異時点間選択は（そして，利潤率に基づく対象選択は）どのように変化するだろうか．

3.3　社会採餌と異時点間選択

ここでは社会採餌によるシナジー効果はなく，一定の餌資源が個体間に配分されるという条件を考える．

(a)　1 頭の採餌者が二つの餌を前にした状況では，どちらを選択しても，採餌者は餌のすべてを得る．

(b)　2 頭の採餌者が 1 つの餌を競う場合，近いほど多くを得る．

(c)　生産者の前には 2 つの餌，後ろには掠奪者が控えている状況では，生産者はより近い餌を選ぶべきだ．近い餌の方が生産者の取り分は大きい．（掠奪による分け前の減少が小さい．）

(d)　掠奪者が 2 頭の生産者を見つけた．この場合も，より近い生産者を襲うべきだ．近い生産者から，より多くを掠奪できる．

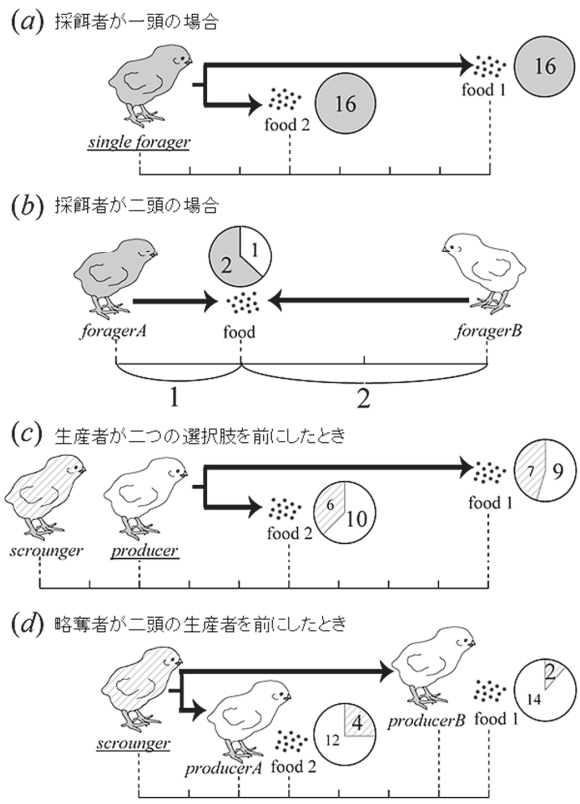


図 5　群れの中における異時点間選択

生産者（producer）と掠奪者（scrounger）という 2 つの戦術を，どの個体も柔軟に使い分けている状況を考えよう．(a) 採餌者が 1 頭だけなら，どちらの餌も独占できる．(b) 餌を 2 頭の採餌者が分け合う場合，取り分は近いほど大きい．(c) 後ろに掠奪者が控えている時，生産者は近い餌を採るべきだ．(d) 前に 2 頭の生産者を見つけた時，掠奪者は近い生産者を襲うべきだ．

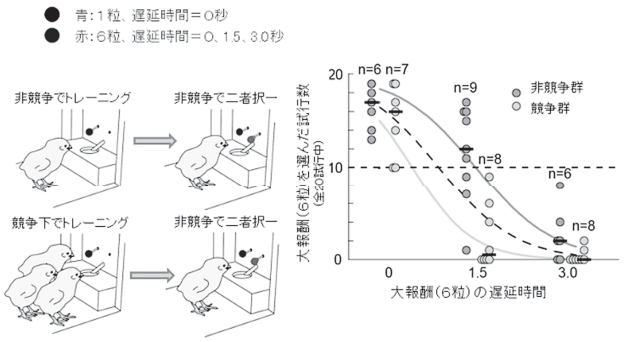
この (c) から，つぎの予想が導かれる．『資源を競争する状況では，より衝動的な採餌者が有利である．』さらに，もし衝動性が変化する形質なら，『競争の経験は，衝動性を亢進する．』他方，(d) からは別の予想が導かれる．『掠奪者は，より近い生産者を襲え．』

同一の個体が生産者・掠奪者の 2 つの戦術を速やかに切り替えるならば，動物たちは互いに衝動性を高め合いながら，他個体へ強く引きつけられ，群れは同調すると予想される．実際のヒヨコは，この予想を満たした（**図 5**）．

4.　ヒヨコを用いた神経行動学

4.1　競争採餌は衝動性を高める

孵化後 3，4 日からヒヨコは活発な採餌を始める．この



$$X = 1.51 + (-1.13) \times delay + (-1.19) \times delay{:}comp + ri$$

一般化線型混合モデルによって選ばれた最良の推定式（AICによる）

図 6　競争採餌と衝動性

青なら，小さな餌がすぐに得られる．赤なら，大きな餌を待たねばならぬ．待ち時間 0 秒の群，1.5 秒の群，3 秒の群を用意した．さらにトレーニング中の競争の有無によって 2 条件を設け，計 6 群の結果を比較した．選択比（縦軸）をロジスティック関数 $(Q(X) = 1/(1 + \exp(-X)))$ によって線形予測子 X に逆写像し，この X の値を遅延時間 $(delay)$ および競争 $(comp)$ を説明変数とする線型関数で最尤推定した．その結果を式として示す．遅延時間の効果（変数 $delay$ の係数）は，競争によってほぼ倍増された．

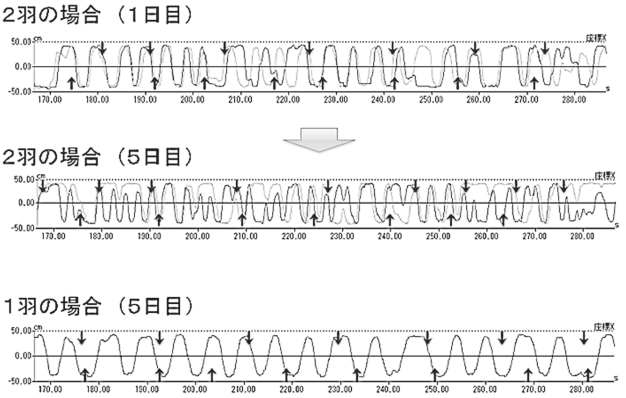


図 7　社会的促進と同調

長さ 80 cm の走路の両端に，給餌装置を 1 つずつ置く．給餌には手がかりを与えず，ヒヨコは 2 つの給餌装置の間を行き来して，餌を回収しなくてはならない．ヒヨコの運動軌跡を 3 例示した．横軸が時間（約 2 分間），縦軸がヒヨコの位置（両端間で 80）である．矢印は給餌の時点を示すが，これは同調因子になっていない．2 羽の軌跡は互いに引き込み合って，1 羽の時より多くの距離を走る．上 2 段は同一の個体の行動を実験の 1 日目と 5 日目で比べたものである．5 日目は，2 羽の離合集散（同期と非同期）が激しく交代している．最下段は対照群で，同一日齢の別の個体を常に 1 羽で行動させた場合の軌跡の例である．

時，群れで競争的にトレーニングすると，高い衝動性（強い時間割引き）が観察されるようになった[14]（図 6）．

競争群と非競争群の間の差異が最も著しいのは，遅延時間を 1.5 秒に設定した場合だった．6 粒の餌の遅延時間を 0 秒とした場合，競争群と非競争群の間に違いはない．競争経験は量の判断に影響はせず，遅延に基づく決定に対して選択的だった．

競争条件のもとでは，当然，ビーズを啄ばむ行為と，餌を得る帰結とが乖離する．3 羽のどの個体がビーズを啄んでも餌は出る．出てきた餌は分け合わねばならず，当然，獲得量は変動する．しかし量の変動は衝動性を亢進しなかった．衝動性を亢進するためには，リスクも不必要だった．さらに，競争採餌はその場の選択には影響しないこともわかった．過去の競争，トレーニング時の経験の積分が，衝動性を高めると考えられる．

線条体（側坐核）の局所破壊でも衝動性は亢進する．図 3B の●は破壊後の選択を示したもので，競争採餌と同様，遅延時間が 0 秒の場合選択に影響はない．大脳線条体を含む神経回路は，量と近さのバランスを決定していると判断される．

実際，競争条件のもとでは，側坐核と近傍（内側線条体）のニューロン活動が修飾を受けている．この領域のニューロンは，予期される餌の量あるいは近さを表現すること[15]が判明しているが，競争はこれを抑制するのである．同じ脳領域には，現実に得た餌をリアルタイム・モニターするニューロンもあるが，競争採餌はこの活動をも抑制する．競争は「ご馳走（予期と評価）を台無しにする」のである[16]．

「台無しにされたご馳走」の脳内表現が，いかに時間割引きを高めるのか，そのメカニズムの全容はまだ解明されていない．今後の課題である．

4.2　採餌エフォートを促進する

採餌に投資する運動量も，競争によって促進された[17]．衝動性亢進と異なり，影響は急性的である．競争の累積を必要とせず，その場の速やかな変化として現れる（図 7）．

走路の両端に給餌装置を置き，低い利益率（平均 15 秒の間隔で 1 粒の粟）で給餌した．手がかりは与えず，ヒヨコは近づいて初めて粟の存在に気付く．この条件のもとで，ヒヨコは活発に往復運動を続けた．この軌跡を約 2 分間分示したのが，図 7 である．1 羽の場合（最下段），ヒヨコはゆっくりと往復を続けるが，給餌の時点（矢印）に同調しない．他方，2 羽の場合（最上段），ヒヨコは互いに主となり従となりながら，左右の餌場を激しく往復した．

なお，2 羽には利益相反が伴う．そこで前節の実験と同様，2 羽のヒヨコを透明なアクリルで仕切って別の餌を与え，利益相反を排除した．この疑似的な競争条件でも，結果は同様だった．むしろ同調はより高い水準を保った．

5．展望

ごく駆け足で，採餌と選択，そして社会の基本的な関係を示した．自然界では餌資源に関する完全な情報を採餌者が獲得することは現実的ではない．さらに他者の出現と行動に依存して，自己の行為が何に帰結するか，これも大きく変わる．そのため個々の採餌者が利益を向上するためには，未来を長期にわたって見通す必要はない．近視眼的に利益を予期推定し，直近の利潤率に対応して対象を選ぶこと，だけで十分である．各個体が最適性を追求する必要はないし，実際に最適性は実現していない．そして，群れの

個体は一様に衝動性を亢進し一様にエフォート投資を増やして，行動を同調させる．

ヒヨコの場合，社会的統制を考えに入れる必要はなかった．しかし，シャチのように個体間の血縁がある場合，また霊長類のように社会的ランクがある場合，これらの個体間相互作用は均衡点をずらすだろう．それでも，割引き率やエフォート量，同調など一連の行動特性は，餌を仲介として群れの中で共有されると期待できる．

個体の行動特性という微視的な特性が変化した時，群れの挙動はどれほどの遅れをもって変化を始めるだろうか．それを明らかにするためには，群れの中の 1 個体の行動特性，あるいは 2 個体の関係に変更を加えたとき，影響が群れに伝搬する速さ，また，伝搬を引き起こす最小の変化量（閾値）を実測する必要がある．また，他の行動形質（たとえばリスク感受性）も共有情報となりうるか，を検討したい．リスク感受性はしばしば時間割引きを究極原因として説明される．共通の認知メカニズムを持つならば，両者はリンクして伝搬するだろう．

ここでは個体の意思決定と 2 個体間の相互作用という，微視的プロセスのみに基づいて，平衡状態における群れの挙動を論じた．今後は，空間分布（餌と個体の不均衡分布）や個体間の血縁（血縁選択）を組み込んで拡張し，時間発展を繰り込んだ動的モデルを構築することが求められる．本稿が共同研究のきっかけとなることを望みつつ，筆をおきたい．

（2012 年 11 月 19 日受付）

参 考 文 献

1） D.W. Stephens and J.R. Krebs: Foraging Theory, Princeton University Press (1986)
2） 粕谷英一：行動生態学入門，東海大学出版会 (1990)
3） 川森愛，松島俊也：リスク感受性の行動生態学—変動する世界における適応的戦略，動物心理学研究（投稿中）
4） E.L. Charnov: Optical foraging, attack strategy of a mantid, *American Naturalist*, **110**, 141/151 (1976)
5） G. Ainslie: Breakdown of Will, Cambridge University Press (2001)
6） R.S. Sutton and A.G. Barto: Reinforcement Learning, An Introduction, The MIT Press (1998)
7） R.J. Herrnstein: The Matching Law, Papers in Psychology and Economics, Harvard University Press (1997)
8） E.-I. Izawa, G. Zachar, S. Yanagihara and T. Matsushima: Localized lesion of caudal part of lobus parolfactorius caused impulsive choice in the domestic chick: evolutionarily conserved function of ventral striatum, *Journal of Neuroscience*, **23**, 1894/1902 (2003)
9） T. Matsushima, A. Kawamori and T. Bem-Sojka: Neuroeconomics in chicks: foraging choices based on amount, delay and cost, *Brain Research Bulletin*, **76**, 245/252 (2008)
10） R.W. Baird and M.D. Lawrence: Ecological and social determinants of group size in transient killer whales, *Behavioral Ecology*, **7**, 408/416 (1996)
11） L.-A. Giraldearu and T. Caraco: Social Foraging Theory, Princeton University Press (2000)
12） R. Noe and P Hammerstein: Biological Markets, *Trends in Ecology and Evolution*, **10**, 336/339 (1995)
13） M.D. Gumert: Grooming and infant handling interchange in Macaca fascicularis: the Relationship between infant supply and grooming payment, *International Journal of Primatology*, **28**, 1059/1074 (2007)
14） H. Amita, A. Kawamori and T. Matsushima: Social influences of competition on impulsive choices in domestic chicks, *Biology Letters*, **6**, 183/186 (2010)
15） E-I. Izawa, N. Aoki and T. Matsushima: Neural correlates of the proximity and quantity of anticipated food rewards in the ventral striatum of domestic chicks, *European Journal of Neuroscience*, **22**, 1502/1512 (2005)
16） H. Amita and T. Matsushima: Long-term modification of choice impulsiveness by competitive foraging and the medial striatum / nucleus accumbens of domestic cihcks, In: *10th International Congress of Neuroethology*, (Abstract) 5/10, August, Maryland, USA (2012)
17） Y. Ogura and T. Matsushima: Social facilitation revisited: increase in foraging efforts and synchronization of running in domestic chicks, *Frontier in Neuroscience*, **5**: article 91 (2011)

［著 者 紹 介］

松島俊也君

1986 年東京大学大学院理学系研究科博士課程修了，理学博士．ブレーメン大学・カロリンスカ研究所・上智大学・名古屋大学を経て，2007 年より現職（北海道大学・理学研究院・教授）．動物行動学と認知脳科学を素直に結び付ける道筋を探しながら，両生類や魚類の研究を続けてきた．今は鳥の行動に焦点を絞って研究している．

Wen Chentao 君

2007 年中国武漢大学応用物理学科卒業．11 年から北海道大学大学院生命科学院（修士課程 2 年次在学中）．大脳基底核のニューロン群が，餌の主観的価値をどのように表現しているのか，これを明らかにするために自由行動中の神経活動を解析している．

小倉有紀子君

2009 年北海道大学理学部生物科学科卒業．11 年北海道大学大学院生命科学院修士課程修了．（現在，博士課程 2 年次在学，日本学術振興会特別研究員．）人間や動物の行動に見られる社会的促進と，その神経メカニズムに関心をもって研究を進めている．

網田英敏君

2008 年北海道大学理学部生物科学科卒業．10 年北海道大学大学院生命科学院修士課程修了．（現在，博士課程 3 年次在学，日本学術振興会特別研究員．）コンフリクトに直面した時の動物の行動と，意思決定の神経メカニズムに関心をもっている．