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Research report

Long-term behavioral sensitization to apomorphine is independent of conditioning and increases conditioned pecking, but not preference, in pigeons

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ABSTRACT

When rodents are given a free choice between a variable option and a constant option, they may prefer variability. This preference is even sometimes increased following repeated administration of a dopamine agonist. The present study was the first to examine preference for variability under the systemic administration of a dopamine agonist, apomorphine (Apo), in birds. Experiment 1 tested the drug-free preference and the propensity to choose of pigeons for a constant over a variable delay. It appeared that they preferred and decided more quickly to peck at the optimal delay option. Experiment 2 assessed the effects of a repeated injection of Apo on delay preference, in comparison with previous control tests within the same individuals. Apo treatment might have decreased the number of pecks at the constant option across the different experimental phases, but failed to induce a preference for the variable option. In Experiment 3, two groups of pigeons (Apo-sensitized and saline) were used in order to avoid inhomogeneity in treatments. They had to choose between a 50% probability option and a 5-s delay option. Conditioned pecking and the propensity to choose were higher in the Apo-sensitized pigeons, but, in each group, the pigeons showed indifference between the two options. This experiment also showed that long-term behavioral sensitization to Apo can occur independently of a conditioning process. These results suggest that Apo sensitization can enhance the attractiveness of conditioned cues, while having no effect on the development of a preference for variable-delay and probabilistic schedules of reinforcement.

1. Introduction

In mammals, striatal dopamine plays a central role in enhancing the attractiveness of rewards and of their conditioned stimuli (CSs), which are predictive of reward delivery [1–5]. This process explains why hungry rats come to approach and nibble a lever CS associated with food, and also why repeated administration of dopamine agonist-like drugs, such as amphetamine and cocaine, may generate addictive behaviors. Indeed, the administration of dopamine agonists causes long-lasting neuroadaptations that gradually stimulate behavioral responding, such as locomotion and gnawing in rodents [6–8].

Dopamine release is more elevated when a reward is obtained after a short rather than a long delay [9–12]. Also, dopamine has been shown to favor the pursuit of a reward obtained with variable rather than constant effort in ratio-schedules [13–16] and to influence choice in probabilistic schedules [17]. However, the information currently available on this topic has only been collected in mammalian species. In this paper, we would like to examine dopamine's role in choice tasks in an avian species, the domestic pigeon.

The dopaminergic system of birds is similar to that of mammals in terms of anatomy and of its behavioral and neuronal effects on working memory [18–22]. However, during 310 million years of separate evolution of birds and mammals, some changes of dopamine receptors and distribution of dopaminergic fibers occurred [23–25]. Does this may have had significant alterations in the way they respond to dopaminergic drugs? Here, we would like to determine whether (i) a drug-induced stimulation of dopamine neurons can increase the pecking rates of pigeons to a CS predictive of food delivery, (ii) their propensity to choose between a CS associated with a variable option and a CS associated with a constant option, and (iii) their preference for the former over the latter option. Finally, (iv) our methodology is appropriate to examine the so-called conditioned effects of behavioral sensitization. These questions, in particular how dopamine can control choice behavior, have received no attention in birds. In pigeons,

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Kalenscher et al. [26] inactivated telencephalic regions containing dense concentrations of dopamine receptors by means of tetrodotoxin (TTX), a potent Na^+ channel blocker. They compared the preference of sham-operated and TTX-injected individuals in a free-choice task (fixed-short versus fixed-long delay), and found that inactivation of the ventral and dorsal striatum inhibited the pecking responses, irrespective of whether the delay between a response and food access was short or long. Inactivation of the nidopallium caudolaterale – the functional equivalent to mammalian prefrontal cortex – increased activity for the long time-interval and decreased it for the short time-interval. While this task tests choices along the time domain, it neither specifically targets the dopaminergic system nor the effects of variability, as studied in mammals. So, the question whether dopamine plays a role in "risky" choice remains open. Other studies reported that pigeons may prefer a random-ratio schedule to a fixed-ratio schedule, but they did not explore the behavioral effects of dopaminergic drugs [27,28]. In this paper, we examined conditioned pecking, the propensity to choose, and preference when pigeons have to decide between a constant and a variable delay or between probabilistic uncertainty and a constant delay, while choice behavior was modified with the non-selective dopamine agonist apomorphine (Apo).

Delius and colleagues showed that repeated systemic injection of Apo strongly increases the number of pecking responses – but not locomotion – in pigeons [29–33]. Under 1 mg/kg Apo injected in the pectoral muscle for several consecutive days, pigeons can provide up to 4000 pecks within 20 min. Such stereotyped responses are typically directed to non-edible, unrewarded small items (e.g. dots), especially when located on the walls rather than on the floor of the cage – only a small proportion of pigeons are floor-peckers. Given the strong appetite-suppressant effect of Apo [34], food items can be grasped but they are not swallowed. Repeated Apo treatment alters dopamine receptor densities [35], and the effects of those neuroadaptations may last for two years [36]. It was predicted that Apo pretreatment will increase conditioned (CS-directed, but not unfocused) pecking, the propensity to peck when exposed to a free choice, and preference for variability.

2. Experiment 1

This experiment aimed to estimate the sensitivity of pigeons to delays, and especially their preference for a fixed or a variable delay. Finding parameters for which variability was *not* preferred to constancy was important for the next experiment, where the hypothesis that Apo can generate a preference for variability was tested.

2.1. Materials and methods

2.1.1. Animals and housing conditions

Twenty one naïve pigeons (*Columba livia*) were maintained at approximately 80–85% of their free-feeding body weight for the duration of the experiment. During the workdays, pigeons received no extra food. Water was freely available in the home environment. The birds were obtained from local breeders and most of them were housed in an aviary – four animals, two in each group, were housed in individual cages because of space limitation (12 h light/dark cycle, light on at 7:30 a.m.). They had been accustomed to their home environment for several weeks before the experiment begins. All procedures followed the German guidelines for the care and use of animals in science, and were in accordance with the European Communities Council Directive 86/609/EEC concerning the care and use of animals for experimentation.

2.1.2. Apparatus

The pigeons were tested in individual operant chambers (34 cm × 34 cm × 50 cm). On the front panel, two transparent pecking keys (4 cm × 4 cm) coupled with electric switches allowed the animal to respond to the 10-s presentations of CSs displayed on an LCD flat screen located behind the panel. The two pecking keys were 17 cm above the floor level of the chamber. In the autoshaping phase, the CS consisted of a white dot (8 mm in diameter) in the middle of a black background. In the forced- and free-choice tasks, other stimuli were used. One CS was an unfilled red triangle with a white background. The other CS was an unfilled red square with a white background. The two CSs were of the same color and provided the same contrast with the background in order to avoid possible chromatic preference for one CS over the other [37]. A tone (1000 Hz) emitted during the first 3 s of the presentation of one or two CSs acted as a signal that the CSs were being presented. A food hopper was centered below the two keys, 5 cm above the floor level, providing an access to small-sized grains for 3 s when it was moved up and preventing their accessibility when it was moved down. On the back panel, a camera allowed the experimenter to observe the pigeon's activity, but video data were not recorded. Each chamber was positioned in a sound-attenuating cubicle. A custom-written MATLAB code (The Mathworks, Natick, MA, USA) controlled the apparatus [38].

2.1.3. Procedure

The pigeons received one daily session of Pavlovian autoshaping (40 trials; ITI 30–90 s) for several consecutive days in order to learn to peck at a CS when presented on a response key. Following the 10 s of the CS presentation, food became immediately accessible for 3 s – even if no pecks were given. Because the two response keys had to be used subsequently, the CS was presented in alternation on the left or the right key from session to session. In each group, half of the pigeons had their first session with the CS presented on the left key, and the other half with the CS presented on the right key. Autoshaping training was continued until each pigeon could reach a mean of 3 pecks per CS presentation over a session. Note that only 9 individuals received autoshaping training, the other 12 individuals had already been trained in a previous experiment based on serial autoshaping. Immediately after, the pigeons started forced-choice training with two new CSs. They were presented on distinct response keys and the key-CS associations were always the same for a given pigeon (Fig. 1). During forced-choice training, a session consisted of 40 trials, interspersed with a 45-s ITI. A trial involved the presentation of a CS for 10 s, and when turned off, food became accessible for 3 s following a delay. The two CSs differed with respect to the delay between the end of the CS presentation and food delivery. A first group of pigeons (group 1–7, n = 12) was trained with one CS predictive of a variable delay of 1 or 7 s and the other CS predictive of a fixed delay of 4 s – equivalent to the variable delay's arithmetic mean. These short delays were selected to avoid the adverse effects of temporal discounting, which are stronger in pigeons than in

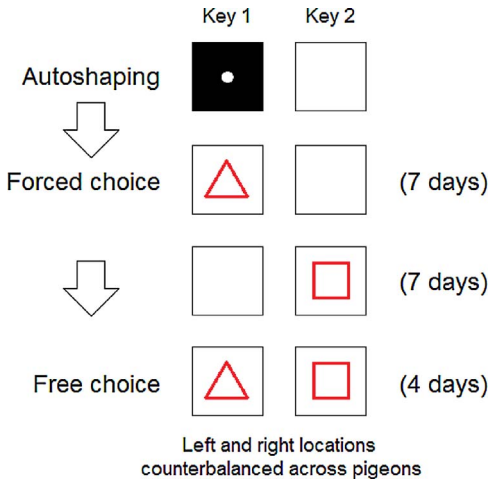

Fig. 1. Procedure used to train and test the pigeons in Experiment 1. The same general method was used to train and test them in the other two experiments.

the members of other species [39,40]. Later, other individuals were trained with an altered variable delay. In this second group (group 2–12, n = 9), one CS was also associated with a fixed delay of 4 s, but the other CS with a variable delay of 2 or 12 s. Here, the variable delay was less favorable because its minimal value was closer to that of the fixed delay (2 s instead of 1 s, relative to 4 s) and provided food after a longer mean time (7 s) than the fixed delay (4 s). Specifically, in each group, the pigeons were trained for five days with one CS, five days with the other CS, and finally with a daily alternation of each CS for four more days. Overall, they received seven sessions with each CS presented alone. They had to peck (at least once) on the illuminated key to be rewarded. Half of the pigeons from each group had their first session with one CS presented on the left key and the other CS presented on the right key, while the other half of the pigeons were exposed to the reverse configuration. This reduced the risk that the pigeons developed an initial preference for one strategy over the other. Also, the CS-delay associations were counterbalanced across pigeons. But the same CS-delay association always appeared on the same key for a given pigeon. During the next four sessions, the pigeons had to choose between the two CSs simultaneously presented, one on each key. The first peck given (selected key) turned the other key off, and the number of pecks was only recorded for the selected key. The same durations as reported above were used for CS presentations, delays, and food access. The amount of food received per session was independent of the option chosen, as a session consisted of a fixed number of trials which were all rewarded. The difference between the two options was that variability was associated with a possibly quicker delivery of food than constancy. After each training session, the animals were returned to their home cage.

2.1.4. Statistical analyses

We used mixed ANOVAs for group comparisons with repeated measures and *t*-tests for related samples. As appropriate, planned comparisons allowed us to examine the differences between two data sets. We used ANOVAs with repeated measures only to compare the amount of pecks on the variable- and/or the fixed-delay keys within the same pigeons. Statistica 12 was used to process the data.

2.2. Results

Autoshaping results showed that the pigeons had correctly learned to peck in response to the presentation of a CS. The number of pecks increased significantly between the first and the last autoshaping day, which could vary from one individual to another (day 1: 6.897 ± 3.109; last day: 18.207 ± 2.278; $t(8) = -3.855$, $p = 0.005$). The pigeons were then trained with the forced-choice trials for 7 days, as reported in Fig. 2A. There was no significant effect of group ($F(1,29) = 0.835$, $p = 0.368$) and no Group × Day interaction ($F(6,174) = 0.500$, $p = 0.807$). A significant effect of Day was obtained ($F(6,174) = 2.660$ $p = p = 0.017$), but it was only visible between Days 1–4 in group 2–12 ($F(1,29) = 8.603$, $p = 0.006$) and between Days 1–8 in group 2–12 for the variable delay ($F(1,15) = 4.589$, $p = 0.049$).

After forced-choice training, the pigeons from both groups were immediately tested in the free-choice procedure. In group 1–7 (Fig. 2B), the number of key selections for the fixed and the variable delays remained similar over the 4-day period (fixed: $F(3,33) = 1.307$, $p = 0.288$; variable: $F(3,33) = 1.278$, $p = 0.298$). On average, the pigeons chose to peck the variable-delay option more than the fixed-delay option, and this preference was significant during the last two days (Day 3: $F(1,11) = 18.417$, $p = 0.001$; Day 4: $F(1,11) = 7.724$, $p = 0.018$). In group 2–12 (Fig. 2C), the pattern of choice was reversed, with a non-significant tendency to peck the fixed-delay option rather than the variable-delay option ($F(1,8)$'s ≤ 2.732, p's ≥ 0.137), and the number of key selections remained stable over the four days under free-choice (fixed: $F(3,24) = 2.207$, $p = 0.113$; variable: $F(3,24) = 0.131$, $p = 0.941$). Finally, the number of omissions (non-response trials) over the four free-choice sessions was examined (Fig. 2D). This number did not change in group 1–7 ($F(1,19) = 0.024$, $p = 0.878$), but decreased in group 2–12 ($F(1,19) = 6.288$, $p = 0.021$). Accordingly, although initially very similar between the two groups (Day 1: $F(1,19) = 0.230$, $p = 0.637$), the number of omissions came to be significantly lower in group 2–12 than in group 1–7 (Day 3: $F(1,19) = 15.560$, $p = 0.001$; Day 4: $F(1,19) = 21.407$, $p = 0.000$).

2.3. Discussion

The pigeons preferred variability (1 or 7 s) to constancy (4 s) in one group and were non-significantly inclined to prefer constancy (4 s) to variability (2 or 12 s) in the other group. This result is in line with the evidence that pigeons are strongly delay-averse [39,40], although variability was not the sole factor that distinguished the two groups. The difference between the smallest variable-delay value and the fixed option, variability range, and arithmetic mean were all more favorable in group 1–7 than in group 2–12. In group 2–12, the less favorable values for the variable delay tended to cause a preference for constancy. The analyses of the number of omissions indicated that the pigeons were perhaps more often undecided to respond when the variable delay had an average value equivalent to that of the constant delay (group 1–7), suggesting that this factor perhaps impairs decision-making more than variability range itself (larger in group 2–12). The fact that the same CS-delay association always appeared on the same key for a given pigeon may have induced a preference for a specific location or strategy at the individual level. But the possible individual preferences were unlikely to interfere with the decisions at the group level, as the key-CS (or key-delay) associations were counterbalanced across pigeons. In addition, our results indicate that the pigeons were strongly sensitive to the experimental conditions; if they had a preference for a specific location or strategy, a reversal of the preference would not have been so easy to obtain.

The pattern of responses in group 2–12 was ideal to start Experiment 2. In this experiment, we examined whether Apo could induce a preference for the variable over the fixed option, as observed with other dopamine agonists in rodents [13,14,17,41].

3. Experiment 2

We tested the behavioral effects of Apo sensitization, before and after an incubation phase, on the preference of pigeons for a variable-delay or a constant-delay option. Although the behavioral effects of incubated Apo have hardly been studied, there is some indications that those effects are long-lasting and may occur independently of conditioning to the environmental context [36,42,43].

3.1. Materials and methods

Nine pigeons (reused from group 2–12) were housed and trained in the same conditions as in Experiment 1.

3.1.1. Drug

Apo hydrochloride, obtained from BioMol (Hamburg, Germany), was diluted in a Ringer saline solution to 0.75 mg/ml (sensitization phase) or to 0.05 mg/ml (challenging dose), and injected with a volume of 1 ml/kg. When saline was used for control purposes, an equivalent volume was injected. It must be noted that the half-life of Apo is short, occurring after about 20 min in the striatal neural tissue of rats [44] and this is similar in pigeons [32].

3.1.2. Cardboard box with carbon papers

Intramuscular Apo in pigeons is known to elicit a high rate of pecking responses [32]. We prepared cardboard boxes (33 cm × 31 cm × 34 cm) to assess the effects of acute and repeated

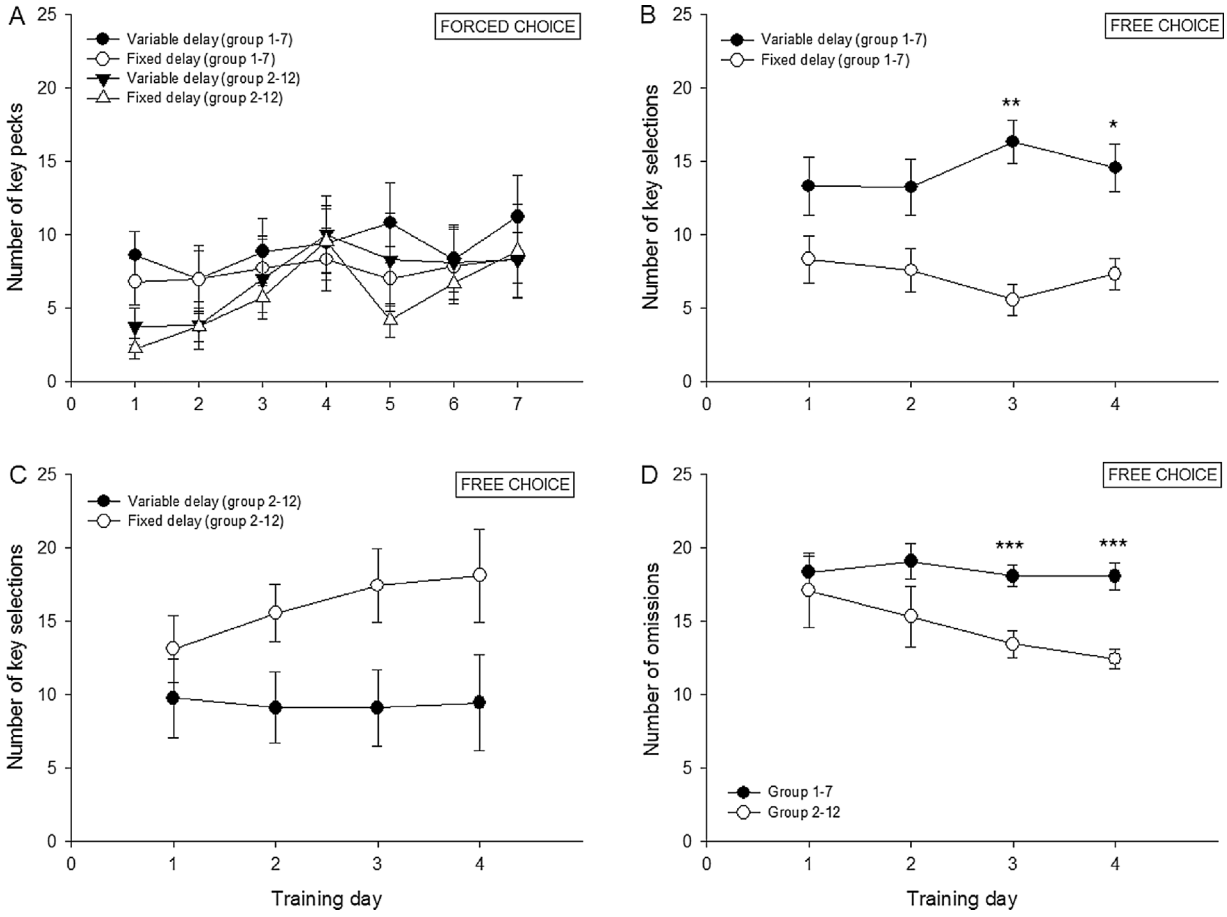

Fig. 2. Experiment 1: Pigeons' behavior in a free-choice task involving a fixed delay (4 s) and a variable delay (1 or 7 s in one group and 2 or 12 s in the other group). (A) Training with each delay type (fixed and variable), presented separately in the two groups. (B) In group 1–7 (n = 12), the pigeons came to select the variable delay more often than the fixed delay. (C) In group 2–12 (n = 9), the reverse tendency was observed: on average, the pigeons were inclined to select the fixed delay more often than the variable delay. (D) During the free-choice sessions, the number of omissions remained constant in group 1–7, but gradually decreased in group 2–12. M ± SE, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Apo injections. A box was open on top to allow for the ambient light to penetrate inside, while a mosquito net prevented the pigeon from flying away. The pecks were recorded by means of carbon papers placed on the four walls and also on the floor. This method was inspired by laboratory works on bird migration and navigation, which recorded the claw marks let by birds using blotting paper and an inkpad [45], typewriter correction paper [46], and more recently thermal paper [47]. In the boxes (called "carbon" boxes thereafter), the pigeons were incited to peck on A4-format white (normal) paper sheets, one per wall and one on the floor, on which small dark gray dots (8 mm in diameter) were represented (approximately 100 per 10 cm^2). Behind each dotted sheet was a carbon paper, and behind it was an A4 white paper sheet without dots, used to collect the carbon-marked pecks (Fig. 3A). The wall sheets were fixed horizontally, about 10 cm above the floor level in order to avoid possible claw marks. Note that we placed a carbon sheet on the floor after noticing that two pigeons (out of nine) pecked only the floor, not the walls. For the remaining pigeons, the floor sheet had no identifiable marks.

3.1.3. Procedure

Immediately after the completion of the four free-choice sessions without injection (Experiment 1), the pigeons were given one more session under saline, administered 2–3 min before testing. Saline (and later, Apo) was injected in the pectoral muscle, half of the total volume on each side. The last day of Experiment 1 (no injection) and the first day of Experiment 2 (saline) were used as baselines for comparisons with post-sensitization days, during which the pigeons were tested without injection or with an Apo challenge. The day after the pigeons received their saline injection, a phase of sensitization to Apo started. Sensitization was induced in a context (carbon boxes) that differed from that in which the pigeons were subsequently tested (Skinner boxes), in order to avoid possible Apo-conditioned effects. To begin with, the pigeons were subjected to a one-day habituation session to the carbon boxes, placed one per box for 30 min without injection. They were exposed to the dotted white sheets but the carbon sheets were not used at this stage. The next day, the pigeons were placed again in the carbon boxes for 30 min after receiving a saline injection for control purposes. Pecks were recorded by means of the carbon sheets. The next day, sensitization to Apo started and it was continued for 8 consecutive days. On each day, the pigeons received an injection of Apo (0.75 mg/kg, i.m.), were placed in the carbon boxes for 30 min, and data were recorded via the carbon sheets. The day after termination of the Apo sensitization phase, the pigeons were retested in the free-choice task, as previously and without injection, for three consecutive days. Doing this, we wanted to assess the immediate effects of Apo sensitization on pecking in animals that were no longer under the influence of acute Apo. Then, the pigeons were subjected to an incubation phase for 10 days in order that the potential effects of the drug on dopamine neurons were maximized. During incubation, the pigeons remained in their home cage without any treatment. After incubation, the pigeons were retested in the free-choice task with a low challenging dose of 0.05 mg/kg for three consecutive days. This low challenging dose aimed to reactivate the possibly sensitized neurons while avoiding appetite suppression. One month later, the pigeons were replaced in the carbon boxes under saline for one session in order to assess the late conditioned effects of Apo. A summary of the procedure is presented in

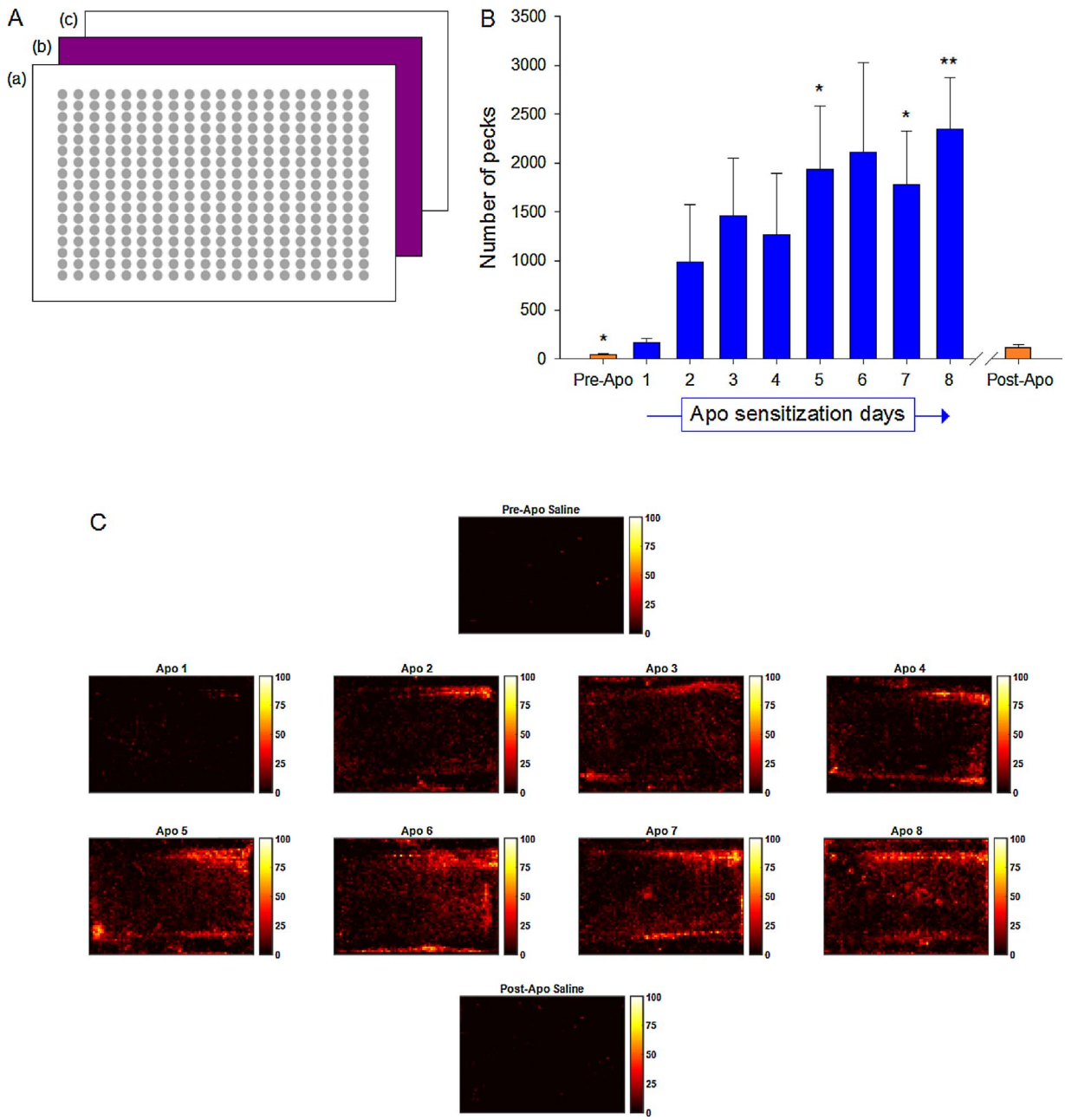

Fig. 3. Experiment 2: Data collected from the cardboard boxes with carbon papers ("carbon" boxes). (A) Method allowing us to record the pecks during the 30-min exposure to a carbon box: (a) dotted-white paper sheet that invited the pigeons to peck, (b) carbon paper, (c) white paper sheet on which the carbon-marked pecks appeared. (B) Sensitizing effects of repeated Apo injection (8 days) on the pecking rates of pigeons (n = 9). The first (Pre-Apo) and the last (Post-Apo) days were saline control days before and after Apo sensitization, respectively. Significant effects were computed relative to Apo day 1 (*$p < 0.05$, **$p < 0.01$). (C) Density distribution of pecks for the two saline control days (top and bottom pictures) and for the 8 days of sensitization to Apo.

Figs. 3B and 4 A.

3.1.4. Statistical analyses

Data collected from the carbon boxes were analyzed by means of a custom-made MATLAB code, allowing us to count the pecks on each paper sheet. Once digitalized, a sheet consisted of an image of 3100 × 2300 pixels. To represent the density distribution of pecks per day, we used the Image Processing Toolbox of MATLAB. Briefly, a Wiener filter (20-by-20 local neighborhood of each pixel) was applied to the data in order to remove artifacts arising from body movements on the carbon sheets. The Wiener filter minimized the overall mean square error, removed the additive noise and inverted the blurring simultaneously. Because the marks let by pecks had a higher density than artifact-induced marks, the images were converted to binary images using an appropriate threshold, which was $T = (Im(x,y) - \min(Im))/(\max(Im) - \min(Im))$, where $Im(x,y)$ is the density of pixels at coordinates (x,y) in the image Im. As a result, the marks with a high probability of being caused by pecking were kept for counting. The ultimate erosion of the binary image was then computed by means of the "bwulterode" function of MATLAB. Finally, the pecking marks were sometimes very close to each other on a sheet. To clarify the digitalized pattern of responses, the regional maxima of the Euclidian distance transform of the binary image was computed. Because one-pixel marks were so small areas, they were assumed to be artifacts rather than pecks. Separate areas were found in the whole image (using the "regionprops" function of MATLAB), and any area bigger than one pixel was counted as a peck. This program was pretested with computer-generated random patterns of dots whose number was known in advance. Some of those patterns

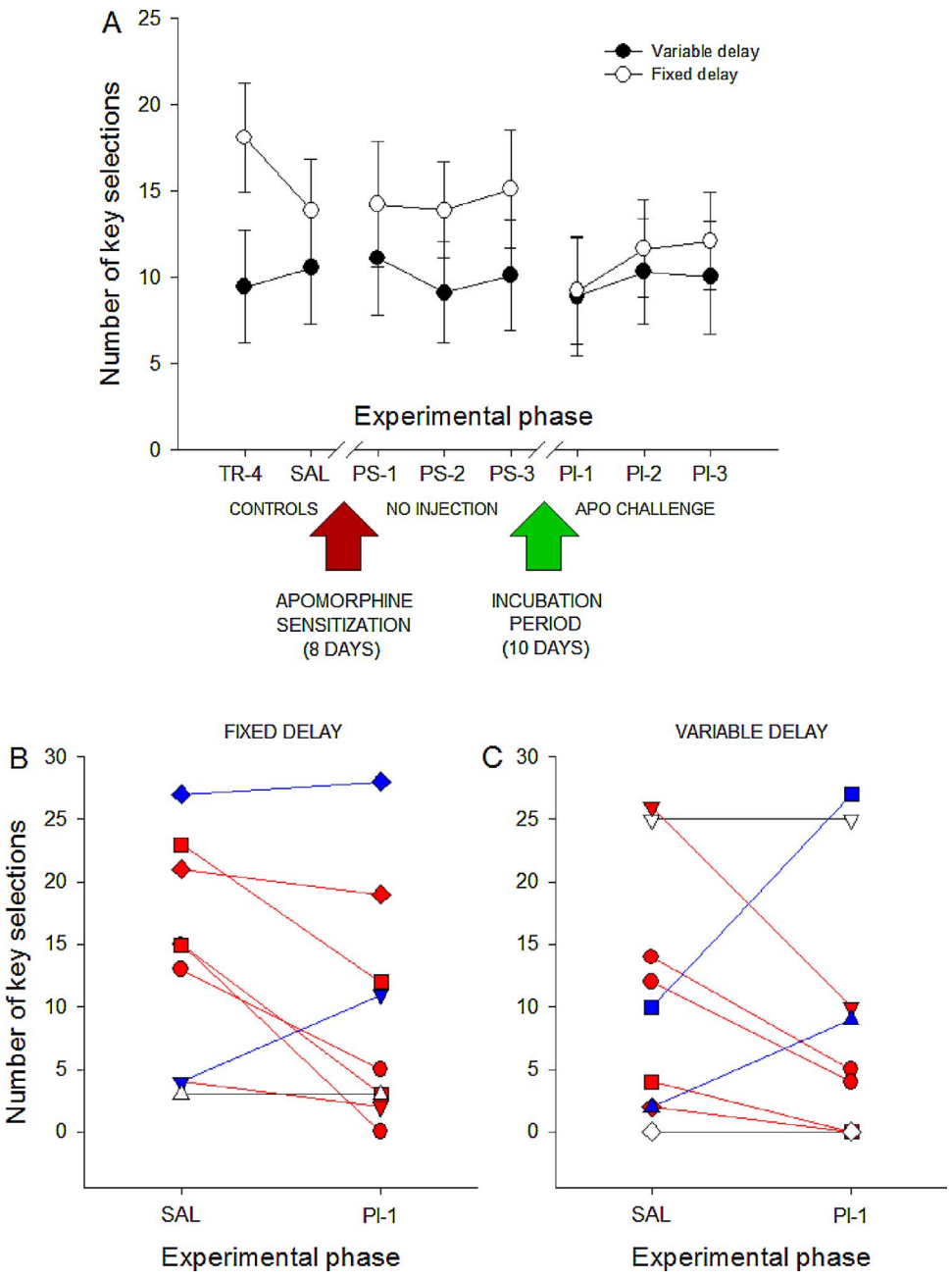

Fig. 4. Experiment 2: The effects of Apo sensitization and incubation on free choice (n = 9). (A) Apo treatment reduced the number of selections of the key associated with the fixed delay, but had no effect on the key associated with the variable delay. TR-4: last free-choice training day for group 2–12 in Experiment 1, SAL: free choice under saline, PS-1 to PS-3: post-sensitization days without injection, PI-1 to PI-3: post-incubation days with an Apo challenging dose. (B) Pigeon-per-pigeon comparison of the pecking responses to the fixed-delay key between the SAL and PI-1 days: Pearson's r correlation was positive and significant (p = 0.042). (C) Pigeon-per-pigeon comparison of the pecking responses to the variable-delay key between the SAL and PI-1 days: Pearson's r correlation was positive and non-significant (p = 0.125). Blue symbols represent the individuals that increased their performance; red symbols depict the individuals that decreased their performance; white symbols are the individuals whose performance remained stable. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were similar to those of the carbon-marked pecks by pigeons. The number of pecks (at the sheets and at the keys) were analyzed by means of repeated measures ANOVAs, *t*-tests for related samples, and Pearson's r correlations, as appropriate.

3.2. Results

3.2.1. Overall effects

Although the different phases of Experiment 2 could not all be properly combined together (because of inhomogeneity in treatments), it is worth mentioning that the progression of those experimental phases had a different effect on the selected (first-pecked) keys (Fig. 4A). The key associated with the variable delay (2 or 12 s) was selected a similar number of times throughout the experiment ($F(7,56) = 0.262$, $p = 0.966$). In contrast, the key associated with the fixed delay (4 s) was selected less and less often as the experiment progressed ($F(7,56) = 2.706$, $p = 0.017$). However, the fixed-delay option was never selected less often than the variable-delay option.

3.2.2. Effect of saline (TR-4 vs. SAL)

A comparison of the last day of initial free-choice test (see Experiment 1, data reported here) with the saline control day revealed no significant differences in responding, irrespective of delay type (variable: $t(8) = -0.637$, $p = 0.542$; fixed: $t(8) = 1.609$, $p = 0.146$). This result indicated that the number of response omissions remained the same.

3.2.3. Sensitization to Apo (carbon boxes)

As depicted in Fig. 3B, repeated Apo injection in the carbon boxes induced a sensitization of the pecking responses over the eight days ($F(7,56) = 2.609$, $p = 0.021$). The number of pecks was significantly lower during the first saline control day (Pre-Apo) relative to the first day of Apo administration ($F(1,6) = 7.922$, $p = 0.030$). Because of the great variability in responding among the pigeons, only the last days (5, 7, and 8) differed significantly from Apo day 1 (d5: $F(1,8) = 7.889$, $p = 0.023$; d7: $F(1,8) = 9.528$, $p = 0.015$; d8: $F(1,8) = 17.526$, $p = 0.003$). Note that the data for two Pre-Apo and two Post-Apo

pigeons were lost.

The rate of responses on the Post-Apo day was intermediate between that observed on the Pre-Apo day (saline) and that observed on the first Apo day, and none of the comparisons were significant (Pre-Post: $F(1,5) = 1.886$, $p = 0.228$; Apo1-Post: $F(1,6) = 0.097$, $p = 0.766$). Accordingly, a strong decrease in responding was obtained between Apo day 8 and the Post-Apo day ($F(1,6) = 18.225$, $p = 0.005$). Fig. 3C shows the distribution and the density of pecks from day to day in the carbon boxes. The gradual sensitization of pecking responses caused by repeated Apo administration can easily be visualized from this qualitative analysis. Also, it clearly appears that a very low amounts of pecks were recorded on both Pre-Apo and Post-Apo days in comparison with the eight Apo days.

3.2.4. Free choice without injection (PS-1 to PS-3)

After the sensitization phase (and before incubation), the pigeons were tested in the Skinner boxes for three days without injection. There was no significant difference between the last drug-free training day and the first sensitization day (TR-4 vs. PS-1), irrespective of delay type (fixed: $t(8) = 1.544$, $p = 0.161$; variable: $t(8) = -0.803$, $p = 0.445$). No differences were also observed across the three post-sensitization days (fixed: $F(2,16) = 0.444$, $p = 0.649$; variable: $F(2,16) = 2.634$, $p = 0.102$).

3.2.5. Free choice with a low challenging dose (PI-1 to PI-3)

After incubation, the pigeons were retested in the Skinner boxes with an Apo challenge (0.05 mg/kg). There was no treatment effect relative to the initial saline injection (SAL vs. I-1) for any delay (fixed: $t(8) = 1.941$, $p = 0.088$, but note the medium effect size, Cohen's $d = 0.51$; variable: $t(8) = 0.522$, $p = 0.616$). A significant decrease in the number of selections of the fixed-delay option occurred between the last training day and the first post-incubation day (TR-4 vs. PI-1; $t(8) = 2.565$, $p = 0.033$), which suggests an effect of incubation perhaps combined with an effect of the injection. No significant changes were observed over the three post-incubation days (fixed: $F(2,16) = 2.119$, $p = 0.153$; variable: $F(2,16) = 0.879$, $p = 0.434$). But key selection for the fixed delay decreased during the three post-incubation days (PI-1 to PI-3) compared with the three post-sensitization days (PS-1 to PS-3; $t(26) = 3.466$, $p = 0.002$). This result also suggests an influence of incubation, possibly combined with an effect of the injection – since no injections were received during the post-sensitization days. A correlational analysis of the change in the individual performances between the saline control day (SAL) and the first post-incubation day (PI-1) supported the hypothesis that incubation had an effect in itself. For the fixed delay (Fig. 4B), there was a positive, significant correlation between the two conditions, suggesting an overall decrease in responding after incubation (Pearson's $r = 0.684$, $p = 0.042$). In contrast, for the variable delay (Fig. 4C), the positive correlation was not significant, indicating that incubation did not influence responding to variability (Pearson's $r = 0.550$, $p = 0.125$).

3.3. Discussion

The strong sensitizing effect of Apo on pecking responses in the carbon boxes revealed that pigeons were sensitive to the drug, as reported in other studies involving pigeons and rodents [7,31,32,48–50]. The effect was observable from the first injection [32,51,52]. Behavioral sensitization to Apo is a long-lasting process [36] and has been assumed to be conditioned, because the contextual cues associated with the drug experience elicit the sensitized response [32,33]. But some results also suggest that neuroadaptations can develop and persist long after initial exposure to Apo. For example, Mattingly and Gotsick [43] showed behavioral sensitization following repeated Apo administration in the absence of drug-associated environmental stimuli, suggesting that non-associative processes play a role in the effects of that drug (see also [42]). In this experiment, we did not test whether behavioral sensitization had resulted in *neural* sensitization after the incubation phase. Thus, referring to neural sensitization to explain these results would only be hypothetical. Also, the absence of conditioned response under saline after 30 days in the Apo-sensitized pigeons may be due to forgetting of the cue-drug association, or perhaps due to the fact that no conditioned response developed. Indeed, we did not perform a saline test in the carbon boxes immediately after the eight days of Apo injection, so it is unknown whether a conditioned response has ever been produced. Whatever the reason, we will test – in Experiment 3 – whether behavioral sensitization in the carbon boxes can persist independently of conditioning in these same pigeons.

The present results show that behavioral sensitization established in one context (carbon boxes) has no effect on choice in another context (Skinner boxes). This supports the view that the effects of Apo are very sensitive to context [32,33]. Across treatments, Apo-sensitized pigeons gradually decreased the number of key selections for the fixed delay, but not for the variable delay. This effect was particularly clear when the pigeons were tested with an Apo challenge after an incubation period. However, the cause of this effect is hard to understand. The inhomogeneity in treatments did not allow us to identify an effect of Apo independently of the injection: Is the small drop in the responses to the fixed delay due to drug incubation, the Apo challenge, or is even accidental? Thus, it is reasonable to think that Apo sensitization was ineffective when tested before or after a 10-day incubation period. More thorough investigations were needed.

4. Experiment 3

In this Experiment, the Apo-experienced pigeons were reused, and each step of the procedure was controlled by means of saline-injected naïve individuals. The Apo pigeons were reused for two reasons. First, it was necessary to determine whether the pigeons were under the influence of some Apo-induced effects when tested in the free-choice task in Experiment 2. Second, we aimed to determine whether context-independent behavioral effects could occur in a new task, after a longer incubation period (approximately two months after the last day of sensitization in Experiment 2). In this new task, the pigeons had to choose between a 50% probability of food delivery and a fixed 5-s delay for the same food (see also [53,54]). The use of a 50% probability is justified by the fact that an option providing an occasional absence of rewards was preferred by rodents under the influence of dopamine agonists [13,14,17].

4.1. Materials and methods

4.1.1. Apparatus

The stimuli were changed in this experiment, in order to avoid previous learning to affect performance among the reused pigeons. One CS was a full blue square with a white background and the other CS was a full blue triangle with a white background. In one option, the pigeons had to associate the CS with a 50% probability of food. So, when the 8-s CS was turned off, food became immediately available for 3 s or remained inaccessible. In the other option, the pigeons had to wait for 5 s after the CS was turned off and food was provided for 3 s with a 100% probability. In this experiment, pecks were not only recorded on the illuminated key, but also on the non-illuminated key, in order to estimate whether pre-sensitized pecking was exclusively directed to the CS or unfocused.

4.1.2. Procedure

The pigeons were trained with the 50% probability and the fixed 5-s delay separately, alternated on a weekly basis and counterbalanced for their respective CS across individuals. Forced-choice training was continued for 10–23 consecutive days, and the last four days consisted of a daily alternation of each option (40 trials per session and ITI 45 s). For the delay option, the time interval was gradually increased from 1,

3, to 5 s over training. The pigeons from group saline (Sal, n = 9) were naïve, so their training phase was longer than for the pigeons from group Apo (n = 9). Forced-choice trials were automatically rewarded after a CS presentation; no independent autoshaping sessions were necessary for the naïve pigeons to learn to peck at the keys. The Apo pigeons started this experiment while already treated with Apo. Immediately after forced-choice training, the pigeons were subjected to five free-choice sessions of 40 trials (ITI 45 s). Up to this point, the pigeons received no injections. During the next three days, the pigeons were tested again in the free-choice procedure, two hours after receiving an Apo injection (group Apo, 0.75 mg/kg, i.m.) or an equivalent volume of saline (group Sal). Specifically, after receiving their injection, the pigeons were placed for 30 min in the carbon boxes, where their pecking responses were recorded (see Fig. 3A), and then they were returned to their home cage for 90 min. They were then exposed to the free-choice task. Given the short half-life of Apo (around 20 min), the remaining amount of drug in their body at the beginning of each session should be very low (≅0.01 mg/kg) − lower than the challenging dose used in Experiment 2.

4.1.3. Statistical analyses

Because the pigeons in group Apo were already accustomed to responding to CSs, the forced-choice training phase in this new task was shorter for them than for the pigeons in group Sal. In order to make comparisons possible, we only showed the responses obtained during the last three forced-choice sessions in each group. Mixed ANOVAs with planned comparisons were used to analyze data from the two groups on different days.

4.2. Results

4.2.1. Forced-choice training

During the last three forced-choice training days, the Apo pigeons pecked the relevant (illuminated) key at a higher rate than the Sal pigeons (Fig. 5A); there was an effect of Group ($F(1,16) = 8.412$, $p = 0.010$) and Day ($F(5,80) = 3.659$, $p = 0.005$), but no Group × Day interaction ($F(5,80) = 2.146$, $p = 0.068$). For each day, the group differences were significant with respect to the 5-s delay ($F(1,16)$'s ≥ 4.856, p's ≤ 0.042) and the 50% probability ($F(1,16)$'s ≥ 6.317, p's ≤ 0.023).

Given the strong discrepancy in responding between groups Apo and Sal on the relevant key, it was important to determine whether responding of Apo pigeons was specific to that key or whether responding was indiscriminative. For that, we recorded the responses provided to the irrelevant (non-illuminated) key during the same training sessions (Fig. 5B). Although the pigeons from both groups pecked the irrelevant key, no significant differences in responding were observed (Group: $F(1,16) = 0.583$, $p = 0.456$; Day: $F(5,80) = 1.524$, $p = 0.192$; Group × Day interaction: $F(5,80) = 1.208$, $p = 0.313$). The number of responses provided here was also very small in comparison with that provided to the relevant key. Also, an examination of the number of omissions indicated that the Apo pigeons pecked the relevant key more often than the Sal pigeons (left part of Fig. 6A; 5-s delay: $F(1,16) = 14.711$, $p = 0.001$; 50% probability: $F(1,16) = 14.590$, $p = 0.001$).

4.2.2. Free-choice test and sensitization to Apo (carbon boxes)

Immediately after forced-choice training with the 5-s delay or the 50% probability, the pigeons from both groups were tested in a free-choice procedure with the same two options presented simultaneously. For five consecutive days, their choice was assessed in the absence of injections. The number of omissions was significantly lower for the Apo pigeons than for the Sal pigeons over the five days (central part of Fig. 6A; $F(1,16)$'s ≥ 8.063, p's ≤ 0.012). Omissions remained stable among the Apo pigeons, while they decreased among the Sal pigeons (d1-5: $F(1,17) = 9.884$, $p = 0.006$). However, omissions remained

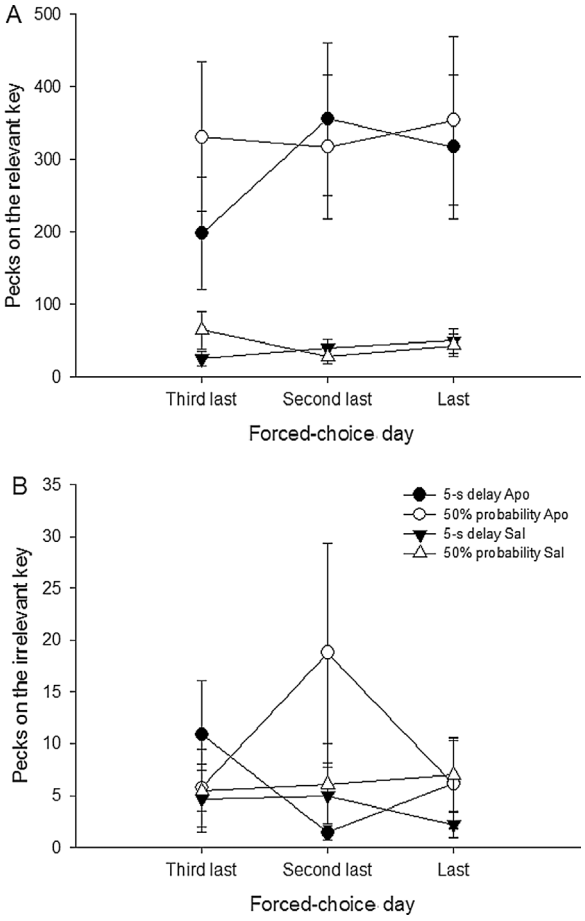

Fig. 5. Experiment 3: Comparisons of the Apo-pretreated and the non-treated pigeons during forced choice training (5-s delay or 50% probability). (A) The pretreated pigeons (group Apo, n = 9) showed a higher response rate on the relevant (illuminated) key than the non-treated pigeons (group Sal, n = 9), both for the delay and the probability. (B) The responses of the pretreated pigeons did not differ from those of the non-treated pigeons on the irrelevant (non-illuminated) key.

stable during the next − and last − three days (right part of Fig. 6A). Here, the Sal pigeons received a saline injection and the Apo pigeons received an Apo injection each day in the carbon boxes, two hours before the free-choice test (see next paragraph). The Apo pigeons slightly increased the number of omissions, perhaps because of residual appetite-suppressant effects of Apo. As a result, the significant group differences observed in the absence of injection were lost for these three days ($F(1,16)$'s ≤ 3.459, p's ≥ 0.081).

When re-exposed to the carbon boxes under Apo (Fig. 6C), the pigeons showed response rates on day 1 almost fivefold over the performance they showed on the very first day of Apo injection in Experiment 2 (large effect size: $\eta_p^2 = 0.33$), and the difference between these two days was close to significance ($F(1,8) = 3.931$, $p = 0.083$). Their response rates were even closer to a significant difference relative to the effects of saline at the conditioning test (Post-Apo day; $F(1,6) = 5.021$, $p = 0.066$), and the effect size was also larger ($\eta_p^2 = 0.45$). During the three days of Apo injection, the pigeons responded in a similar way ($F(2,16) = 0.044$, $p = 0.957$), and the saline pigeons gave virtually no pecks at all (Fig. 6E).

The propensity of pigeons to choose one option over the other was examined (Fig. 6B). In both groups, the pigeons selected the 5-s delay and the 50% probability options in a similar fashion (group Apo: $F(1,16) = 0.349$, $p = 0.563$; group Sal: $F(1,16) = 0.391$, $p = 0.540$). However, note that the delay was selected more often than the probability the first day of injection in group Apo (day 6; $F(1,16) = 6.781$, $p = 0.019$). The omnibus comparisons indicated an effect of Group (F

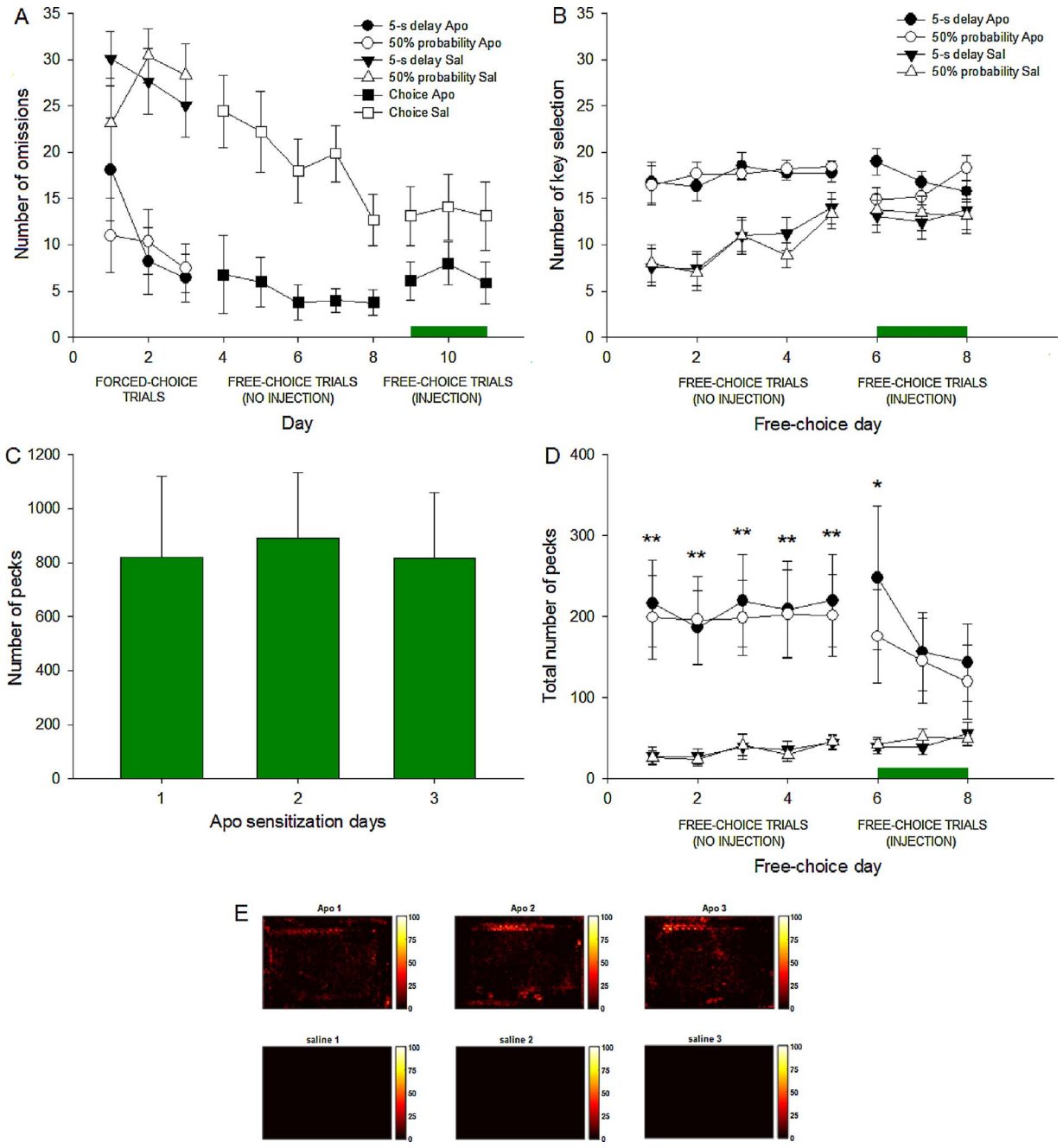

Fig. 6. Experiment 3: Comparisons of the Apo-pretreated and the non-treated pigeons in the free-choice task (5-s delay vs. 50% probability). (A) The number of omissions was lower for the Apo-pretreated pigeons compared to the non-treated pigeons during the forced-choice and the free-choice sessions in the absence of injection. But their response rates were similar during the free-choice sessions with an injection (challenge) − represented by a green line. (B) Accordingly, the Apo-pretreated pigeons selected a key more often than the non-treated pigeons during the free-choice sessions, but there was no preference for delay or probability in any group. (C) Data from the Apo pigeons re-exposed to the carbon boxes before the last three free-choice sessions (see green lines in the other graphs). Apo caused a number of pecks that was similar over the three-day period, while the control pigeons exposed to the carbon boxes under saline did not peck at all (not shown). (D) Before re-exposure to the carbon boxes, the Apo-pretreated pigeons consistently gave a greater total number of pecks at the two keys (selected and non-selected) than the Sal pigeons. After re-exposure, their performance decreased. (E) Density distribution of pecks for the Apo and the Sal pigeons for each day. M ± SE, *p < 0.05, **p < 0.01. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(1,16) = 15.374, p = 0.001) and Day (F(15, 240) = 2.710, p = 0.001), and a Group × Day interaction (F(15,240) = 2.907, p = 0.0003). As the average number of key selection (delay and probability) for an individual is a direct function of the number of omissions for that individual, the statistical values reported in Fig. 6A (central and right parts) were repeated here: the significant group differences observed during the first five days (without injection), were lost during the last three days (with injection). A closer look at the data showed that group differences were present for delay and probability on some days (d1-4; delay: F(1,16)'s ≥ 11.166, p's ≤ 0.004; probability: F(1,16)'s ≥ 32.256, p's ≤ 0.000), delay only (d6; F(1,16) = 6.732, p = 0.019) or probability only (d5 and d8; F(1,16)'s ≥ 5.381, p's ≤ 0.034). Finally, an analysis of the total number of pecks to the selected and the non-selected keys during the free-choice sessions, indicated that the Apo pigeons consistently pecked more often than the Sal pigeons (Fig. 6D; Group: F(1,16) = 8.341, p = 0.011; Day: F(7,112) = 1.327, p = 0.244). In both groups, the pigeons pecked the two keys in a similar fashion. During the five days without injection, as well as the first day of injection, all group differences were significant (F(1,16)'s ≥ 9.159, p's ≤ 0.008). But statistical significance

disappeared during the last two days of injection (F(1,16)'s ≤ 4.393, p's ≥ 0.052).

4.3. Discussion

The response rates of the Apo pigeons in the carbon boxes were elevated in comparison with their performance on the very first day of Apo injection and on the Post-Apo day (late conditioning test) in Experiment 2. This indicates that Apo-induced neuroadaptations were present in the brain of the pigeons throughout Experiments 2 and 3. As already mentioned, we do not provide evidence for or against the development of neural sensitization, so whether or not this mechanism played a role is only an assumption here. Another possibility is that Apo treatment led to neuronal rearrangements through an increase in neuronal plasticity (M. Acerbo, pers. com.). But two important points are that (i) these effects of Apo persisted despite the absence of conditioned effects in the carbon boxes (Fig. 6C) and (ii) gave rise to context-independent effects in the Skinner boxes (Fig. 6B and D). More investigation is necessary to determine whether these context-independent effects are due to prolonged incubation (two months), the use of a new task (probability vs. delay), or the occasional of non-rewared trials (50% chance in one option).

During forced-choice training, the pre-experienced pigeons responded to CSs more often than the non-experienced (saline) pigeons. In the two groups, the responses were focused rather than indiscriminate since very few pecks were given to the irrelevant key. The Apo pigeons also showed a greater propensity to choose between the two options compared to the Sal pigeons. In the free-choice task, the Apo pigeons selected a key more often than the Sal pigeons, although there was no evidence in any group that Apo induced a preference for reward uncertainty − measured in terms of probability. This absence of effect was unlikely to be due to the strong interactions between Apo and glutamatergic and serotoninergic neurotransmissions, leading to appetite suppression [34,35,55], because it was also visible when pigeons had no drug in their body. As discussed further, it is possible that choice involving delay or probability is relatively insensitive to dopaminergic activity [9]. However, our data indicates that even a very low dose of Apo had some appetite-suppressant effects since a noticeable, though small, decrease in responding was shown during the last two days. At this dose (< 0.2 mg/kg), it is unlikely that the Apo pigeons were stimulated to peck (e.g., [32]).

It is noticeable that the free-choice performance of the Sal pigeons became more and more similar to that of the Apo pigeons over time (Fig. 6A and B). Could this result from the fact that the Apo pigeons (already used in Experiments 1 and 2) were more experienced with the setup than the Sal pigeons (which were naïve at the beginning of Experiment 3)? Although an adjustment period was perhaps necessary, it must be noted that the Sal pigeons seemed to have reached a plateau in performance, which did not change during the last four days. Also, the total number of pecks (Fig. 6D) indicates that the Apo pigeons consistently pecked more than the Sal pigeons, at least in the absence of injection, irrespective of reward contingency. The data were collected from the selected and the non-selected key during the 8-s of the CS presentation. Here, it is important to note that "non-selected" does not mean "irrelevant" (as with forced choice), because the initial selection of that key would have been rewarded.

5. General discussion

We set out to find that repeated Apo administration increases the pecking rates of pigeons to CSs predictive of food delivery, their propensity to choose between a variable and a constant option, as well as their preference for the variable option. First, we found context-independent behavioral effects of repeated Apo administration (in the Skinner boxes) only after a prolonged incubation period (two months) in pigeons exposed to a new conditioning task. These effects consisted of (i) a higher number of pecks at the food-predictive key CS during forced choice, (ii) a higher number of pecks at both keys (selected and non-selected) during free choice, and (iii) a greater propensity to peck at a CS than saline controls. Second, no effect of repeated Apo administration on preference for variability occurred. Third, repeated Apo treatments altered context-specific responding (in the carbon boxes) independently of conditioning.

5.1. Motivation and choice

Dopamine agonists are known to facilitate approach behavior and physical contact with CSs, because they stimulate brain reward circuits, which control incentive motivational processes [1,56]. Their repeated administration causes long-lasting sensitization of dopamine neurons, responsible for supernormal CS attraction. In this study, there was no verification that neural sensitization to Apo developed. And current evidence suggests that Apo alters the brain in a different way. For example, the addictive power of Apo is very weak compared to drugs such as amphetamine and cocaine in humans [57,58]. Also, in pigeons, Apo-induced pecking varies inversely to food magnitude, suggesting that it is unrelated to the opportunity to get food [59]. Our results indicates that very low doses of Apo in the body reduce CS-directed pecks, while the injection of many dopamine agonists have the reverse effect. Of course, certain doses of dopamine agonists such as cocaine may reduce appetite [60], but whether non-anorexigenic, addictive doses of Apo exist is unclear. For these reasons, the long-term brain effects of Apo may be different from neural sensitization. Apo might cause neuronal rearrangements through an increase in neuronal plasticity (M. Acerbo, per. com.), which could facilitate pecking more than increasing food-related motivation.

Is the propensity to choose related to impulsivity? Although some authors report a causal relation between the consumption of dopaminergic drugs and motor impulsivity [61], our experiments did not allow us to conclude anything about that. Impulsivity means that individuals prefer a small immediate reward over a larger delayed reward [62]. It also means that individuals are unable to inhibit irrelevant responses in a go/no-go task [63]. Our experimental procedures were different. Of course, impulsive individuals may be more likely to respond than less impulsive individuals when they are given the opportunity to do it. But the fact of responding more often in a free-choice task under Apo does not reflect greater impulsivity in itself. In contrast, this measure (propensity to choose) is compatible with the appetitive-pecking hypothesis: Apo-experienced pigeons have a high motivation to peck, especially when the CSs are predictive of food. So they peck more often at a CS presented alone (forced choice) and also more often when two CSs are presented simultaneously (free choice).

Variable-interval reinforcement schedules facilitate the development of habitual response patterns, leading to considerable more responses than fixed-ratio schedules [64–67]. This tendency was not shown here. In Experiment 1, small changes in delays could easily alter free-choice preference, and the alteration occurred so quickly that the development of habitual responses was unlikely. In Experiment 2, more extensive exposure to the variable vs. constant delays did not induce any preference for one or the other option. This result may seem to be at odds with other findings, but we think it is not. A variable interval does not necessarily generate more responses than a fixed-ratio schedule, if the interval is adjusted in order to render both options equivalent − also called indifference point (for a description of the procedure, see [68]). We did not try to reach an indifference point from pigeon to pigeon, but group 2–12 in Experiment 1 was, on the whole, indifferent to both options. And indifference was maintained in Experiments 2 and 3, irrespective of the injection of Apo. In the absence of an indifferent point, an important question to ask is: how can variable-interval reinforcement schedules give rise to habits? Our results suggest that it is because variability offers the opportunity to receive some food more rapidly. Variability is therefore more attractive than constancy in a

competitive situation (free choice, see Fig. 2B), although variability is not necessarily in the absence of competition with constancy (forced choice, see Fig. 2A).

Most studies in which dopamine caused a preference for variability involved a dopamine D2/D3 receptor agonist (pramipexole or ropinirole), and/or individuals exposed to variable- vs. fixed-ratio schedule conditions [13,17,41,69–71]. Contrary to these studies, our experiments failed to report evidence for an effect of Apo on preference for a variable over a constant option. Given that Apo has an action on any kind of dopamine receptors and that animals are often inclined to prefer variable over constant delays [72], a plausible conclusion is that choice involving delays is insensitive to dopaminergic activity. In support of this hypothesis, Day et al. [9] showed in rats that phasic dopamine release in the nucleus accumbens was higher for a 0-s delay than for a 5-s delay during forced-choice trials. But dopamine signals for both options were identical during free-choice trials; choice was independent of dopamine release. This suggests that dopamine is involved in the coding of reward delay in rats, but that it plays no role when these animals have to decide between two distinct delays. Choice could be based on a cognitive evaluation of the situation, such as the opportunity to obtain food sooner as a rational choice, rather than based on the motivation associated with the quicker food delivery. The absence of motivational component in choice should particularly be expected when all the trials of a session are rewarded, like in our delay-related tasks. This might be more difficult to explain in the case of probability, because not all the trials are rewarded. However, conditioned inhibition for the non-rewarded trials tends to dissipate over training with probabilistic schedules [73], so that both options can come to be equivalent in terms of attractiveness.

If correct, this may mean that the frequent preference for variable over constant delays [72] is insensitive to dopaminergic activity in the brain. More thorough investigation is needed to bring a satisfactory answer to this problem. Ratio-schedules are different because the trials are rewarded only occasionally and the amount of food obtained may depend on how hard the animal works within a limited interval of time. Accordingly, animals tend to respond more to ratio- than to interval-schedules of reinforcement [74].

5.2. Sensitization and conditioning

We showed that long-lasting behavioral effects of Apo sensitization on pecking responses can occur independently of conditioning. Such a result contradicts findings suggesting that the sensitized response to a drug is conditioned, that is, elicited by the environmental cues that surrounded the animal when repeatedly exposed to the drug [30,32,33,75–77,78]. For example, Wynne and Delius [33] trained the same pigeons under Apo in one context and under saline in another context on alternate days (six time in each context). Then, these pigeons were injected with saline in both contexts on alternate days (three times in each context). They found that pigeons pecked significantly more in the context associated with Apo than in the context associated with saline, and concluded that Apo-sensitized responding was conditioned. However, their data do not fully support this interpretation; they only show that sensitization is a context-specific process. If sensitized pecking was conditioned, a decrease in performance would have occurred under saline in the Apo context − because of the absence of the Apo-induced unconditioned response. However, Wynne and Delius's [33] data do not indicate any change in performance between the last training day and the conditioning test. Specifically, the acute effect of Apo (training day 1) caused an average of 250 pecks, approximately. On the last day (training day 6), the pigeons pecked about 380 times. So the expected pecking rate on the first day of conditioning test was: 380–250 = 130 pecks, on average. However, it was also about 380 pecks. This means that a large part of pigeons' responding was not due to conditioning, but rather due to Apo-induced changes in the pigeons' dopaminergic systems, resulting in enhanced conditioned pecking in the presence of appropriate contextual cues.

The reasons why our pigeons showed a strong decrease in their pecking rates under saline (Post-Apo saline day) in the carbon boxes are unknown. A first hypothesis is that conditioned responding did not develop at all, but this is not demonstrated because no saline test was conducted immediately after the last Apo sensitization day in Experiment 2. A second hypothesis is that conditioned responding developed, but that the drug-cue association was forgotten due to the relatively long period of time (± 30 days) without exposure to the carbon boxes between the end of the sensitization phase and the conditioning test. This period of time was much shorter in other studies (e.g., 2 days for Braga et al. [79]; 3 days for Wynne & Delius [33]). As a result, the box-associated contextual cues were no longer able to elicit the appropriated response. Our results are insufficient to decide whether Apo sensitization was partly conditioned or not at all. But the forgetting of the box-associated cues may have suppressed responding, even if sensitization was not conditioned. In Experiment 3, the higher response rates of the pigeons prior to their re-exposure to the carbon boxes (see Fig. 6D), as well as the carbon-box data (see Fig. 6C), indicate that Apo-induced changes in the pigeons' brain were still active and that their expression was independent of the sensitization context.

The role of conditioning in behavioral sensitization to drugs is controversial, and it is not here the place to make a decision on this matter. But it is important to point out that excitatory conditioning fails to explain it very often [80,81]. First, a sensitized response may occur without additional conditioned response. For example, it is observable in animals injected with cocaine under anesthesia [82], and the extinction or inhibition of a conditioned response does not prevent the expression of the sensitized response [83–85]. Mattingly and Gotsick [43] showed that behavioral sensitization to Apo can develop after repeated treatments in the absence of drug-associated contextual cues. More recently, Braga et al. [78] obtained context-specific sensitization effects on locomotion without conditioned drug effects in rats injected with low doses of Apo. Second, the sensitized and conditioned responses may show qualitative and quantitative differences. For instance, the diameter of rotations exhibited by rats tested under cocaine differs from that of rats exposed to contextual cues previously associated with cocaine administration [86]. And the conditioned response is of smaller magnitude than predicted by the excitatory conditioning model [87–90]. Finally, a conditioned response may occur without sensitized response. Haloperidol, a dopamine D2 receptor antagonist, decreases locomotor sensitization to cocaine while letting the conditioned response intact [91]. Also, eticlopride, a dopamine D2 receptor antagonist, prevents the development of locomotor sensitization induced by the dopamine D2/D3 receptor agonist, 7-OHDPAT, but this has no effect on the expression of the conditioned response [79]. These examples do not call the Pavlovian origin of the conditioned response into question, but simply suggest that the mechanisms of its occurrence are different from (and independent of) those of behavioral sensitization. In some situations, the conditioned and sensitized responses are acquired and expressed together, while in other situations they can be dissociated.

6. Conclusion

Our predictions that Apo pre-treatment would increase conditioned pecking and the propensity to choose in a free-choice task were confirmed, but these context-independent effects were only observed in a new conditioning task, after a prolonged incubation period. However, the prediction that Apo pre-treatment would generate a preference for variability over constancy was not confirmed. We think that choice involving delay-related and probabilistic schedules, as opposed to ratio schedules, might be insensitive to dopaminergic manipulations. Although Apo has distinct behavioral effects in pigeons and rodents, our results suggest that the stimulation of dopamine neurons may have similar motivational effects relative to the impact of CSs in delay-related

and probabilistic choices. More thorough investigation is needed to fully understand this phenomenon.

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