Behavioral Sensitization to Apomorphine in Pigeons (*Columba livia*): Blockade by the D₁ Dopamine Antagonist SCH-23390

Martin J. Acerbo and Juan D. Delius
Universität Konstanz

Repeated administration of apomorphine leads to a context-dependent pecking response sensitization. Previously sensitized pigeons (*Columba livia*) challenged with saline in the same context show a conditioned response (CR). The authors studied the effects of intrastriatal injections of the dopamine (D₁) antagonist SCH-23390 on both the sensitized response and the CR. When coadministered with apomorphine, SCH-23390 inhibited the initial response to apomorphine, prevented the development of sensitization, and impaired the maintenance of an already developed sensitization. However, SCH-23390 had no effect on the retrieval of a previously established CR. It is concluded that the activation of D₁ receptors in the caudal avian striatum is necessary for the acquisition and maintenance of the sensitization, but not for the expression, of the CR.

In birds, a medium dose of apomorphine elicits a prolonged bout of repetitive pecking, and almost no other activities (Brunelli, Magni, Moruzzi, & Musumeci, 1975; Cheng & Long, 1974; Delius, 1985; Machlis, 1980). In pigeons, this pecking is highly similar to forage pecking and is in fact facilitated by food deprivation (Siemann & Delius, 1992; Wynne & Delius, 1995). Nevertheless, it is not normally directed at grains, as apomorphine has, somewhat paradoxically, a strong hunger-suppressing effect (Deviche, 1984). It is instead aimed at small, inedible items or features (Keller & Delius, 2001). Repeated daily injections of a given dose of apomorphine yield a substantial pecking response increase, up to a dose-dependent asymptotic level (Delius, 1985; Godoy, 2000). This sensitization effect bears similarities to the sensitization that is obtained in rodents with psychostimulant drugs such as amphetamine and cocaine. These animals also exhibit a progressive increase of the mainly locomotor response elicited when these drugs are repeatedly injected. Because it seems possible that the sensitization relates to the addictive properties of these drugs, the phenomenon has been much researched. Nevertheless, the details of the processes that bring about sensitization are still debated (Adams, Careri, Efferen, & Rotrosen, 2000; Anagnostaras & Robinson, 1996; Crombag, Badiani, Maren, & Robinson, 2000; Hinson & Poulos, 1981; Kalant, 1989). The lack of agreement might stem from the fact that cocaine and amphetamine are two differently acting, indirect, and unspecific dopamine agonists (Bedingfield, Calder, & Karler, 1996; Laudrup & Wallace, 1999). Some direct and specific agonists of dopamine such as quinpirole and

apomorphine, although not known to be addictive, also give rise to behavioral sensitization. In rodents, however, these substances elicit a number of different stereotyped reactions and locomotor responses that apparently index somewhat different sensitization variants (Battisti, Uretsky, & Wallace, 1999, 2000; Mattingly & Gotsick, 1989; Mattingly, Koch, Osborne, & Gotsick, 1997; Moller, Nowak, & Kuschinsky, 1987; Tirelli & Heidbreder, 1999; Willner, Papp, Cheeta, & Muscat, 1992). Earlier on, it was considered that the sensitization might be directly caused by a sensitivity increase in the dopaminergic mechanisms mediating the locomotor activity, or that they were indirectly caused by a gradual habituation to (or familiarization with) the procedures and environments involved (Stewart & Badiani, 1993; Stewart & Vezina, 1991).

Concerning the sensitization of pigeons to apomorphine, we have found these explanations wanting. Instead we have proposed a more satisfactory account based on a classical, Pavlovian conditioning process. Apomorphine acts as an unconditioned stimulus (US) that elicits an unconditioned pecking response (UR). When the pigeons repeatedly experience the apomorphine effect in a particular experimental cage, this context functions as a conditioned stimulus (CS_{cage}, but see below) that promotes the development of a conditioned incremental pecking response (IR). The waxing IR adds to the initial UR to constitute a sensitized response (UR + IR). This agrees with the finding that the pecking IR is expressed only in the same cage environment in which the pigeons previously experienced the apomorphine effects. Control treatments have indicated that this context dependency is unlikely to be due to a nonassociative habituation to the relevant cage and procedures (Godoy & Delius, 1999; Keller, Delius, & Acerbo, 2002; see also later in the present report). Furthermore, when pigeons that had been sensitized to apomorphine in an experimental cage were challenged afterward with saline in the same cage, they showed a conditioned pecking response (CR; Keller & Delius, 2001; Lindenblatt & Delius, 1987; Wynne & Delius, 1995; see also below). This CR, however, amounts to only a fraction of the sensitization IR. Smaller CRs relative to IRs are in fact also often observed in amphetamine and cocaine sensitization experiments

Martin J. Acerbo and Juan D. Delius, Allgemeine Psychologie, Universität Konstanz, Konstanz, Germany.

This research was supported by research grants from the Deutsche Forschungsgemeinschaft, Bonn, Germany. Martin J. Acerbo received a Landesgraduiertenstipendium, Baden-Württemberg. We thank Ines Krug and Jennifer Lee for manifold assistance.

Correspondence concerning this article should be addressed to Martin J. Acerbo, who is now at the Department of Psychology, Biopsychology Program, East Hall Building, University of Michigan, Ann Arbor, MI 48109-1109. E-mail: mjaacerbo@umich.edu

(cf. Anagnostaras & Robinson, 1996). In pigeons, the difference is explained by the fact that systemically administered apomorphine, besides functioning as a US, undoubtedly is also in evidence as an interoceptive stimulus (Delius, Krug, Leydel, Keller, & Acerbo, 2004; Djamoz & Wagmer, 1992; Järbe, 1984). We assume that this cue acts as a CS_{apo} and the sensitization IR is really driven by a roughly multiplicative $CS_{cage} \times CS_{apo}$ compound ($CS_{cage \times apo}$; Delius, Acerbo, Keller, & Godoy, 2001; see also Bouton, 1993). Upon saline challenges, the CS_{apo} component is obviously missing, and so the CR obtained is only a minor fraction of the IR (Godoy & Delius, 1999; Keller & Delius, 2001). Upon apomorphine challenges in a cage other than that used for the preceding sensitization (effectively a CS_{cage} -absent condition), one might expect to obtain a corresponding part response to the isolated CS_{apo} component. However, this response is necessarily elusive because it is only a minor additive to the far stronger pecking UR triggered by apomorphine through its US quality. It is fair to mention that similar, though mostly not quite as radical, conditioning accounts have also been proposed for the sensitization to amphetamine and cocaine obtained in rodents (Anagnostaras, Schallert, & Robinson, 2002; Crombag et al., 2001; Tirelli, 2001; Zavala, Nazarian, Crawford, & McDougall, 2000).

We hypothesize that a neural mechanism analogous to that suggested by Wickens (1990) to explain sensorimotor learning might be involved in generating the context-dependent sensitization to apomorphine. This author proposed that the dopaminergic nigro/tegmento-striatal projections converge with glutamatergic corticostriatal pathways in the ventral striatum and interact synaptically, so that a nearly simultaneous activation of both these pathways strengthens the glutamate-mediated transmission. In sensitization, the dopamine agonists presumably mimic the activation of the former pathway and trigger stereotyped responses (the US-UR link). The $CS_{cage \times apo}$ compound is supposed to activate some of the latter glutamatergic pathways. These sensory pathways are assumed to be modulated at earlier relay stages (e.g., the retina; Djamoz & Wagmer, 1992) by dopaminergic synapses on which apomorphine can also act (affecting the CS_{apo} component in our case). The near-simultaneous activations strengthen the relevant glutamatergic synapses of the cortico-ventro-striatal projections and mediate the development of the CS-CR link. Although by no means undisputed, this kind of neural model of context-dependent sensitization has recently gained some support (Bell, Duffy, & Kalivas, 2000; Kelley, 1999; see also Acerbo, Gargiulo, Krug, & Delius, 2002).

Dopamine is known to bind to five different metabotropic receptors. The receptors that induce the formation of cyclic adenosine monophosphate (cAMP) are grouped into a D_1 -like family (D_1 , D_5) and those that inhibit the cAMP synthesis are grouped into a D_2 -like family (D_2 , D_3 , and D_4 ; Keabian & Calne, 1979; Sealfon & Olanow, 2000). In the above-mentioned model, it has been assumed that dopamine acts solely on D_1 -type receptors. However, that may not apply to the sensitization-related learning that concerns us here. Earlier authors have established that the UR pecking acutely induced by apomorphine is mainly triggered by the stimulation of D_2 -type receptors, but that they are potentiated by the stimulation of D_1 -type receptors (Osuid & Adejoh, 1973; Zarrindast, Hajian-Heydari & Hoseini-Nia, 1992; see also later). At the doses in which apomorphine reliably elicits oral stereotyp-

ies in rodents, it can also be assumed to strongly activate both types of receptors (Baldessarini, Kula, Zong, & Neumeyer, 1994; Waddington & Daly, 1993). It is not at all clear, however, which role the two types of dopamine receptors play in the sensitization to psychostimulants. Although, for example, we have evidence that the D_1/D_2 receptor ratio in the striatum of pigeons is augmented after sensitizing apomorphine treatments (Acerbo, Výboh, Kostal, Kubiková, & Delius, 2004), Richtand, Kelsoe, Kuczenske, and Segal (1997) found no alterations in messenger RNAs that code the D_1 - and D_2 -type receptor proteins in the striatum of rats after sensitizing amphetamine treatments.

The aim of the present study was to evaluate the adequacy of the above neural model by finding out what effects SCH-23390, a dopamine antagonist known to block D_1 -type dopamine receptors (Bourne, 2001; Hilditch, Drew, & Naylor, 1984; Hyttel, 1983) might have on the acquisition, maintenance, and retrieval of the sensitization to apomorphine in pigeons. Because the high cost of SCH-23390 precluded a systemic application, and because of the special role that the caudal striatum seems to play in the context of conditioning and sensitization (Amalric, Ouagazzal, Baunez, & Nieoullon, 1994; Caine, Heinrichs, Coffin, & Koob, 1995; Robbins & Everitt, 2002), the D_1 antagonist was microinjected into this particular brain area. In birds, a core structure of the telencephalon, the paleostriatum primitivum, is surrounded by a larger structure, the paleostriatum augmentatum. In the earlier literature, the former was equated with the mammalian globus pallidus and the latter with the mammalian putamen and caudatus (Dubbledam, 1998). More recently, the paleostriatum primitivum has been considered equivalent to the pallidal subdivision, and the paleostriatum augmentatum together with the lobus paraolfactorius has been considered equivalent to the dorsostriatal subdivision, of the basal ganglia of mammals (Durstewitz, Kröner, & Güntürkün, 1999). In any case, the caudal paleostriatum augmentatum receives massive dopaminergic inputs from the medial part of the substantia nigra compacta. These inputs are associated with high densities of both D_1 and D_2 receptors. The source of a sparser dopaminergic input into paleostriatum primitivum is not known, the densities of D_1 and D_2 receptors being markedly lower there (Durstewitz et al., 1999).

We examined whether the coadministration of SCH-23390 in addition to apomorphine would affect the acquisition of the IR, and if so, whether it would also alter the subsequent expression of the CR. We assessed separately whether the elicitation of a previously established CR would be affected by the administration of SCH-23390. Because some studies have indicated that a chronic administration of dopamine antagonists can lead to a hypersensitization to dopamine and its agonists (Lappalainen, Hietala, Pohjalainen, & Syvalahti, 1992; Randall, 1985; Schwartz, Greenwald, Fletcher, Houle, & DaSilva, 2003; Severson, Robinson, & Simpson, 1992), we also checked whether repeated SCH-23390 injections might have such an effect. We additionally examined whether SCH-23390 coadministration had any effect on an already sensitized pecking response to apomorphine. When discussing the results, we refer to two further studies in which we evaluated the effects of a predominant D_2 -type receptor antagonist (haloperidol) and of an NMDA glutamate receptor antagonist (MK-801; Acerbo, Godoy, & Delius, 2003; Acerbo, Lee, & Delius, 2004).

Method

Subjects

Drug-naïve domestic pigeons (*Columba livia*), bred from local homing stock and weighing between 450 and 550 g, were used. A week before the experiments began, they were moved from an outside aviary to individual stainless steel grid cages (45 cm long $\times$ 45 cm wide $\times$ 45 cm high) located in a well-ventilated, brightly lit room with a 12:12-hr light–dark cycle. Except during a preliminary experiment (see below) the birds had free access to water, grain, and grit. Animal maintenance and treatments conformed to the standards and regulations of the German animal welfare laws.

Surgery

Before the pharmacobehavioral procedures began, all pigeons were bilaterally implanted with cannulas into the caudal striatum. Pigeons were anesthetized with an initial intramuscular (im) injection of 0.1 ml Kemint (100 mg/ml ketamine chlorhydrate; Alvetra, Neumünster, Germany) and 0.02 ml Rompun (23 mg/ml xylazine; Bayer, Leverkusen, Germany) per 100 g body weight. The anesthesia was maintained with additional 0.1-ml injections of Kemint at 25-min intervals. The pigeon's head was held in a stereotaxic apparatus (Stoelting, USA) while two guide cannulas (stainless steel tubing, 23 gauge, 11 mm long) were inserted, with their beveled tips aimed at locations 2 mm above the caudal pole of the paleostriatum primitivum surrounded by the paleostriatum augmentatum, at the stereotaxic coordinates A 7.5, D 6.5, L 3.0 from the Karten and Hodós (1967) pigeon brain atlas. The cannulas were fixed to the skull with acrylic cement and kept occluded with removable stainless steel pins (30 gauge, 11 mm long). The pigeons were allowed at least 1 week to recover after surgery.

Drugs

SCH-23390 (R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) was obtained from Tocris-Cookson (Bristol, UK) in crystalline form. It was dissolved in saline with 0.1% dimethyl sulfoxide (Merck, Germany) added for solubility. Solutions with 5, 3, and 1 $\mu\text{g}/\mu\text{l}$ SCH-23390 were prepared. Volumes of $2 \times 1 \mu\text{l}$ of either one or the other of these solutions were bilaterally injected intracerebrally (ic). Equivolumes of $2 \times 1 \mu\text{l}$ saline with 0.1% dimethyl sulfoxide were administered for control purposes. For these injections, the occlusion pin of the relevant cannula was removed and a 30-gauge stainless steel injection cannula connected to a microsyringe was inserted through the guide cannula. The length of this latter cannula (13 mm) was adjusted to reach the caudal striatum at the above-specified coordinates. The 1 μl volume per site was injected gradually over a 2-min period, and the injection cannula was left in place for an additional 1 min to allow diffusion.

Apomorphine hydrochloride, obtained from Teclapharm (Lüneberg, Germany) as a ready clinical solution (10 mg/ml), was diluted with saline to a 1 mg/ml solution just prior to injection. In earlier studies, we established that doses between 0.2 and 2.0 mg/kg im (pectoral muscle) apomorphine yield an orderly, increasing set of dose-dependent sensitization curves (Basten-Kreff, 1977; Godoy, 2000). Throughout this study, we used a 0.5-mg/kg im dose of apomorphine. Equivolume im doses of saline were administered for control purposes; details are given below. The ic injections were always given first and the im injections second.

In exploratory trials, we observed that the ic administration of $2 \times 1 \mu\text{g}$ and $2 \times 5 \mu\text{g}$ SCH-23390 slightly inhibited and totally blocked, respectively, the pecking induced by the standard im apomorphine dose. With the higher dose, the pigeons showed signs of marked sedation. Dopamine antagonists generally, and SCH-23390 particularly, are known to induce sedation at higher doses (Lublin, Gerlach, & Peacock, 1993; Motles, Gomez, Tetas, & Gonzales, 1993). In a preliminary experiment we used 3 pigeons with on-target injection sites (see Figure 1) that were food de-

prived to 80% of their normal body weight. They were first injected with $2 \times 1 \mu\text{l}$ ic saline on 3 days and then with $2 \times 3 \mu\text{g}$ ic SCH-23390 on a further 4 days. After the injection procedures were complete, the pigeons were returned to their home cages and provided with a trough containing a mixture of millet and grit. Their pecks were counted for the 5 min following the first peck into the mixture. The pigeon's mean pecking rate of approximately 900 pecks/5 min was unaffected by the SCH-23390 treatment, and the birds showed no overt signs of sedation. We thus adopted the $2 \times 1 \mu\text{g}$ SCH-23390 as a low dose and the $2 \times 3 \mu\text{g}$ SCH-23390 as a moderate dose.

General Procedure

For the main experiments, immediately after being injected (see below for details), the pigeons were individually placed into distinctive experimental cages. These were standard cages which had their inner back and side walls lined with white cardboard panels randomly sprinkled with dark-green dots (0.8 mm diameter, approximately 10 per 100 cm²). The distinctive cages were located in a separate, brightly lit room equipped with a video camera and recorder. In there, the pigeons were videotaped for 20 min before they were returned to their home cage. The videotapes were later reviewed, and the number of pecks per session were counted with a tally counter. Pigeon pecks involve quite distinct, easy-to-recognize motion patterns (Hörster, Krumm, Mohr, & Delius, 2002) that permit accurate counts, which yield interobserver agreement coefficients of $r_s > .85$. Preening responses that occasionally occurred in lieu of pecking when SCH-23390 was coadministered with apomorphine were disregarded (see also below). The experimental schedules to which the various groups of pigeons were subjected consisted of two successive phases, almost always involving two different cotreatments. The abbreviated www + xxx/yyy + zzz -type names given later to the experimental groups allude to these successive treatments, where the initial www and yyy terms indicate ic saline or SCH-23390 administrations and the following xxx and zzz terms indicate im saline or apomorphine administrations (for details see below). Mean pecking scores with standard errors were computed for each group and each day. Because these data were often not normally distributed, all statistical analyses were carried out with nonparametric Mann–Whitney *U* (between-group comparisons) and Wilcoxon's *T* (within-group comparisons) tests.

Sensitization and Conditioned Pecking

Four experimental groups were formed with 43 cannulated pigeons. For the first 6 days, the sal + apo/sal + sal group pigeons were injected with saline ic and apomorphine im, sch1 + apo/sal + sal group pigeons were injected with the low SCH-23390 ic dose and the apomorphine im dose, and the sch3 + apo/sal + sal group pigeons were injected with the moderate SCH-23390 ic dose and the apomorphine im dose. For a further 3 days, all three groups were injected with control saline ic and saline im doses. The sal + apo/sch3 + sal group pigeons were injected with saline ic and apomorphine im for the first 6 days, and then with the moderate SCH-23390 ic dose and saline im for a further 3 days. After the injections, the pigeons were videorecorded.

Pretreatment and Sensitization Maintenance

Three experimental groups were formed with another 28 cannulated pigeons. For the first 6 days, the sch3 + sal/sal + apo group pigeons were injected with moderate SCH-23390 ic and saline im, then for a further 6 days with saline ic and apomorphine im, and finally for another 3 days with saline ic and im. For the first 6 days, the sal + apo/sch3 + apo group pigeons were injected with saline ic and apomorphine im, and then for 4 days with the moderate SCH-23390 ic dose and the apomorphine im dose. For the 6 first days, the sal + apo/sal + apo group pigeons were injected

Figure 1. Injection locations for the various groups of pigeons are described in the text. For simplicity, all sites are mapped onto 7.5 mm anterior left-side brain section drawings (Karten & Hodos, 1967) even though half of them were actually located on the right-side spread, and the actual site locations spread between 0.5 mm more posterior and 0.5 mm more anterior. Sal = saline; Apo = apomorphine (0.5 mg/kg); Sch1 = 1 μ g SCH-23390; Sch3 = 3 μ g SCH-23390; N = neostriatum; PP = paleostriatum primitivum; PA = paleostriatum augmentatum; CA = commissura anterior; A = archistriatum. Reprinted from Harvey J. Karten and William Hodos. *A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)*. pp. 78–79, Fig. A 7.5. Copyright 1967 Johns Hopkins University Press. Reprinted with permission of The Johns Hopkins University Press.

with saline ic and apomorphine im, and then for a further 4 days again with saline ic and apomorphine im. After the injections, the pigeons were videorecorded.

Histology

After the experiments had been completed, the participating pigeons were anesthetized and injected ic with $2 \times 1 \mu$ l of 0.1% (wt/vol) cresyl violet solution. Then they were perfused transcardially with saline and 4% (wt/vol) formalin in phosphate-buffered solution. The brains were removed and postfixed in the formalin solution, with 30% (wt/vol) sucrose added for at least 1 day. They were then blocked and sectioned (40 μ m) with a cryotome. The tissue block was inspected with a 10 $\times$ magnifying lens, and every fifth section around the injection site was mounted. The injection

sites were located with a microscope using dark-field illumination and transferred to standard brain section drawings taken from the pigeon brain atlas (Karten & Hodos, 1967).

Results

Histology

The injection sites of most pigeons were located within the target area, the caudal striatum. A few pigeons that had at least one injection site misplaced were excluded from the evaluations (see below). Figure 1 shows the on-target sites of the pigeons belonging to the various experimental groups.

Sensitization Acquisition and Conditioned Pecking

Four pigeons were excluded because their injection sites were off-target. The videorecordings further revealed that 5 pigeons coadministered SCH-23390 + apomorphine spent more than half of their sessions preening. Because this behavior interfered with the quantification of their pecking response, they were also excluded. The results concerning the remaining birds are shown in Figure 2. The mean ($\pm$ SEM) pecking responses shown by the sal + apo/sal + sal group pigeons ($n = 10$), the sch1 + apo/sal + sal group pigeons ($n = 7$), and the sch3 + apo/sal + sal group pigeons ($n = 6$) during Day 1, representing an estimate of the URs, were 349 ± 120 , 731 ± 231 , and 17 ± 16 pecks per 20 min, respectively. The differences were not significant, although that between the sal + apo/sal + sal and sch3 + apo/sal + sal groups was barely so. The response of the latter group on Day 1 is in fact significantly lower (U test, $p < .05$) when compared with the Day 1 response of the sal + apo/sal + sal and sal + apo/sch3 + sal groups combined (443 ± 109 pecks per 20 min). The response increments (IRs) from Day 1 to Day 6 for the same three former groups were $2,467 \pm 177$; $1,030 \pm 462$; and 659 ± 394 pecks per 20 min, respectively. The IRs of both SCH-23390 + apomorphine-treated groups were significantly lower than that of the sal + apo/sal + sal group (U tests, $ps < .05$). Thus, the ic administration of SCH-23390 inhibited the acquisition of an IR to the drug in a dose-dependent manner. In accordance with their identical treatment during this phase (Days 1–6) the mean responses of the sal + apo/sch3 + sal ($n = 11$) and sal + apo/sal + sal groups were closely similar.

The right section of Figure 2 shows the mean pecking response of the groups during the second phase. The CRs estimated by the

Day 7 responses of the sal + apo/sal + sal and the sal + apo/sch3 + sal groups were not significantly different from each other (note the large standard error affecting the mean of the former group) and were both in fact comparable to the CRs shown by apomorphine/saline-treated groups in our other studies (Acerbo et al., 2003; Acerbo, Lee, & Delius, 2004). The Day 7 CRs of the sch1 + apo/sal + sal and the sch3 + apo/sal + sal groups did not differ significantly between them, but both were significantly lower than the CR shown by the sal + apo/sal + sal group (U tests, $ps < .05$). The responses of all these three groups diminished (extinction) over Days 8 and 9 and were then statistically indistinguishable. The results indicate that although the development of the IR to repeated im apomorphine administrations was impaired by the coadministration of ic SCH-23390, the retrieval of a CR, once the IR to apomorphine had been normally acquired, was no longer significantly impaired by the administration of ic SCH-23390.

Pretreatment Effects and Sensitization Maintenance

The data of 3 pigeons were discarded because their ic injection sites were off-target. The results concerning the remaining birds are shown in Figure 3. For comparison purposes, the figure repeats the results of the sal + apo/sch3 + sal group from the previous experiment. This group and the sal + apo/sch3 + apo ($n = 8$) and sal + apo/sal + apo ($n = 9$) groups all showed very similar sensitization courses from Day 1 to Day 6 when all 3 were receiving an im apomorphine treatment. The sch3 + sal/sal + apo ($n = 8$) group that received ic SCH-23390 plus im saline treatment during this phase exhibited almost no pecking. During Days 7–10, the sal + apo/sal + apo group, which continued receiving ic saline

Figure 2. Sensitization acquisition and conditioned pecking. Mean ($\pm$ SEM) number of pecks per 20 min. Left: Days 1–6 of the sal + apo/sch3 + sal (triangles), sal + apo/sal + sal (circles), sch1 + apo/sal + sal (diamonds), and sch3 + apo/sal + sal (squares) groups injected with intracerebral (ic) saline and intramuscular (im) apomorphine (first two groups) or with the low (1 μ g) or moderate (3 μ g) SCH-23390 ic and apomorphine im. Right: Days 7–9, when the first group received moderate SCH-23390 ic and saline im and the last three groups received saline ic and saline im. Note the different response scales applying to the left and right sections of the graph.

Figure 3. Pretreatment effects and sensitization maintenance. Mean ($\pm$ SEM) number of pecks per 20 min. Left: Days 1–6 of the sal + apo/sch3 + apo (squares), sal + apo/sch3 + sal (triangles; this group is repeated from the first experiment for comparison), sal + apo/sal + apo (circles), and sch3 + sal/sal + apo (diamonds) groups. The first three groups were treated with intracerebral (ic) saline and intramuscular (im) apomorphine (0.5 mg/kg), and the last group was treated with moderate (3 μ g) ic SCH-23390 and im saline. Right: Days 7–10 (Days 7–11 for sch3 + sal/sal + apo group), when the groups were treated with the moderate ic SCH-23390 dose and the standard im apomorphine dose, with the moderate ic SCH-23390 dose, and with im saline and im apomorphine (two last groups). Note that the same response scale applies to both the right and left sections of the graph.

plus im apomorphine increased its pecking activity somewhat, but not significantly. The pecking of the sal + apo/sch3 + apo group decreased significantly from Day 6 to Day 7 (T test, $p < .01$) and again from Day 7 to Day 10 (T test, $p < .05$). Nevertheless, the response of this group on Day 7 was appreciably higher than that on Day 1, an estimate of its UR to apomorphine (T tests, $p < .01$). The group's response decayed (extinguished) significantly over the next days (T test, $p < .05$). The sal + apo/sch3 + sal group, as described earlier, exhibited much weaker pecking responses on Days 7–10 while being treated with ic SCH-23390 and im saline.

The sch3 + sal/sal + apo group, which was now being treated with ic saline plus im apomorphine, on Day 7 showed a significantly stronger response than the sal + apo/sch3 + apo and sal + apo/sal + apo groups had shown pooled on Day 1 (U tests, $p < .05$). Afterward, however, from Days 8–12 (Day 12 not shown in Figure 3), its sensitization course did not differ significantly from that of these latter groups. Furthermore, on Days 13–15 (not shown in Figure 3), while being treated with ic saline and im saline, the pecking activity of this group did not differ significantly from that shown by the sal + apo/sal + sal group from the previous experiment during its equivalent extinction phase. The most salient result of this experiment was that when ic SCH-23390 was coadministered with im apomorphine to pigeons previously treated with im apomorphine alone, it depressed their sensitized pecking and progressively led to an extinction of the IR.

Discussion

Neural Substrates of Dopaminergic Pecking

There is some agreement that a dopamine and glutamate interaction within the striatum is necessary for the acquisition of

sensorimotor learning and also for the sensitization to psychostimulant drugs. The reward-signaling dopaminergic mesencephalo-striatal pathway acting upon striatal D_1 receptors is thought to be essentially involved in these processes (Bell et al., 2000; Kelley, 1999; Wickens, 1990). On this basis, we chose the pigeon's caudal striatum, a presumed avian brain equivalent to the dorsolateral striatum of mammals (Dubbledam, 1998), as the site for the ic administration of SCH-23390. The results reported justify this choice. But we naturally cannot exclude the possibility that other brain sites might also be involved in the D_1 receptor-mediated control of the pecking and the sensitization elicited by apomorphine. Indeed, in another study we found that ic apomorphine injected into the shell of the nucleus accumbens, but not into the caudal striatum, elicited pecking (Acerbo et al., 2002; see also Delius et al., 2001). However, at least in mammals, it is known that there are interconnections between the ventral and dorsal striatum (for a review, see Nakano, Kayahara, Tsutsumi, & Ushiro, 2000). If similar intra-striatal connections are also present in birds, they could account for the effects of SCH-23390 administration into the caudal striatum in the present experiments. It is possible that whereas the shell of the nucleus accumbens is sufficient, the caudal striatum is not sufficient but still necessary for the apomorphine-induced pecking and sensitization of pigeons. However, this issue merits further research.

Sensitization Acquisition and Conditioned Pecking

The results of the first experiment's sch3 + apo/sal + sal group indicates that the moderate ic SCH-23390 dose significantly blocked the initial expression of the pecking UR to apomorphine (Day 1), and that it also largely blocked the subsequent emergence

of a sensitization IR upon repeated apomorphine administrations (Days 2–6, Figure 2). This inhibitory effect presumably led to the fact that the CR apparent on Day 7 in the sal + apo/sal + sal group was strongly attenuated in the sch3 + apo/sal + sal group. Although the low ic SCH-23390 dose used with the sch1 + apo/sal + sal group seemed to have no effect on the UR (this needs to be viewed with some caution because of the reduced number of subjects, see above) and only mildly inhibited the development of the IR, it nevertheless led to a significant suppression of the CR. Thus the dopamine D₁ receptor antagonist SCH-23390, while only partly blocking the sensitization to apomorphine, still markedly inhibited the emergence of the pecking CR in response to CS_{cage}. It may be noticed that even though the IR shown by the sch1 + apo/sal + sal group on Day 7 was markedly stronger than that exhibited by the sch3 + apo/sal + sal group, the CR exhibited by both groups is nevertheless statistically indistinguishable. The results from the sal + apo/sch3 + sal group indicated no significant inhibition on a previously established CR by SCH-23390. An intrastratial administration of SCH-23390 seems thus capable of interfering with the development of the IR and CR, but not with the actual retrieval of an already extant CR. These findings accord well with the hypothesis that the sensitization to apomorphine—that is, the development of the IR—is due to conditioning (see the introduction) and that, although the conditioning process is dependant on dopaminergic D₁-type mechanisms, the retrieval of the CR so acquired is no longer dependent on D₁-type striatal transmission. In a previous study, we found that the predominantly D₂-type receptor antagonist haloperidol prevented the expression of the UR and impaired the acquisition of the IR and CR, but did not affect the retrieval of an already established CR (Acerbo et al., 2003). Elsewhere, we have instead shown it to be likely that both the development of the IR and the retrieval of the established CR are in fact dependent on glutamatergic transmission mechanisms (Acerbo, Lee, & Delius, 2004). It thus seems probable that the activation of both D₁- and D₂-type receptors by apomorphine are critical for the development of the IR and the CR, but not for the retrieval of an already existent CR elicited by CS_{cage}.

Pretreatment Effects and Sensitization Maintenance

In the second experiment, the relatively strong responding of the sch3 + sal/sal + apo group at the beginning of the second phase (Day 7) might indicate that the extended pretreatment with the dopamine antagonist SCH-23390 induced a mild hypersensitivity to the dopamine agonist apomorphine (cf. Kostrzewa, 1995; Randall, 1985; Schwartz et al., 2003). However, an alternative, simpler explanation could be that the preexposure to the distinctive cage had enhanced the pecking UR to apomorphine shown in the same cage. Godoy and Delius (1999) found that a mere saline pretreatment in the distinctive cage, that is, a previous familiarization to it, had a mild latent facilitatory effect on the initial response to apomorphine. However, as reported earlier, when SCH-23390 was coadministered with apomorphine, it had a suppressing effect on the pecking response induced by apomorphine. The sal + apo/sch3 + apo group results showed that when SCH-23390 was first coadministered with apomorphine on Day 7 to pigeons previously sensitized to apomorphine it immediately diminished their pecking response (compare with the sal + apo/sal + apo group). The repetition of the ic SCH-23390 plus im apomorphine treatment led

to a near-abolishment of the pecking response on Day 10. A similar course of events was obtained in an analogous experiment using the dopamine D₂ antagonist haloperidol (Acerbo et al., 2003). There, the course of events was interpreted as being due to the retention of the IR accompanied by the blockade of the UR and followed by a gradual extinction of the IR caused by the operation of a CS_{cage} × apo-no US-like condition. This interpretation necessitated the assumption that the CS_{apo} component was mediated by the activation of D₁-type receptors. We believe that we can retain that assumption here, inasmuch as the intrastratially administered SCH-23390, although undoubtedly capable of blocking the US effect of apomorphine, is unlikely to have blocked its CS_{apo} effect. As discussed in the introduction, this effect in the main most probably originates in dopaminergic synapses of the retina (Djamos & Wagmer, 1992).

Conclusion

The present results confirm that, as found by Osuide and Adejoh (1973) and Zarrindast et al. (1992), D₁-type dopamine receptors are involved in the pecking UR evoked by apomorphine. Accordingly, the D₁-type receptors are also involved in the acquisition of the sensitization IR to apomorphine. We postulate that the IR is driven by a compound CS_{apo} × CS_{cage}. The present results suggest that the interoceptive CS_{apo} component occasioned by the drug is not mediated by D₁-type receptors located in the caudal striatum but is likely to be mediated by such receptors elsewhere. Inasmuch as the D₁-type receptor blocker SCH-23390 does not inhibit the retrieval of the CR that is driven by the CS_{cage} component, we can assume that the sensitization to apomorphine is not primarily based on modifications of a D₁-type receptor-based transmission. In another study, we argued that the sensitization is similarly unlikely to be primarily based on modifications of a D₂-type receptor-based transmission (Acerbo et al., 2003; but see Acerbo, Vyboh, et al., 2004). Rather, as supported by the results of a third study (Acerbo, Lee, & Delius, 2004), it is likely that the sensitization to apomorphine in pigeons is based on a modification of glutamatergic transmission mechanisms.

References

- Acerbo, M. J., Gargiulo, P. A., Krug, I., & Delius, J. D. (2002). Behavioural consequences of nucleus accumbens dopaminergic stimulation and glutamatergic blocking in pigeons. *Behavioural Brain Research*, 136, 171–177.
- Acerbo, M. J., Godoy, A. M., & Delius, J. D. (2003). Haloperidol blocks the emergence, but not the expression of a conditioned sensitization to apomorphine. *Behavioural Pharmacology*, 14, 631–640.
- Acerbo, M. J., Lee, J. M., & Delius, J. D. (2004). Sensitization to apomorphine, effects of dizocilpine NMDA blockade. *Behavioural Brain Research*, 151, 201–208.
- Acerbo, M. J., Vyboh, P., Kostal, L., Kubíková, L., & Delius, J. D. (2004). Repeated apomorphine administration modifies relative dopamine D1 and D2 receptor densities. Manuscript submitted for publication.
- Adams, J. U., Careri, J. M., Efferen, T. R., & Rotrosen, J. (2000). Conditioned locomotor stimulant effects of cocaine in rats do not result from interference with habituation. *Psychopharmacology*, 151, 13–18.
- Amalric, M., Ouagazzal, A., Baunez, C., & Nicoullon, A. (1994). Functional interactions between glutamate and dopamine in the rat striatum. *Neurochemistry International*, 25, 123–131.
- Anagnostaras, S. G., & Robinson, T. E. (1996). Sensitization to the

- psychomotor stimulant effects of amphetamine: Modulation by associative learning. *Behavioral Neuroscience*, 110, 1397–1414.
- Anagnostaras, S. G., Schallert, T., & Robinson, T. E. (2002). Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology*, 26, 703–715.
- Baldessarini, R. J., Kula, N. S., Zong, R., & Neumeier, J. L. (1994). Receptor affinities of apomorphine enantiomers in rat brain tissue. *European Journal of Pharmacology*, 254, 199–203.
- Basten-Kreff, A. (1977). *Apomorphin-induziertes Verhalten bei Tauben* [Apomorphine-induced behavior in pigeons]. Doctoral thesis, Ruhr-University, Bochum, Germany.
- Battisti, J. J., Uretsky, N. J., & Wallace, L. J. (1999). Sensitization of apomorphine-induced stereotyped behavior in mice is context dependent. *Psychopharmacology*, 146, 42–48.
- Battisti, J. J., Uretsky, N. J., & Wallace, L. J. (2000). Importance of environmental context in the development of amphetamine- or apomorphine-induced stereotyped behavior after single and multiple doses. *Pharmacology Biochemistry and Behavior*, 66, 671–677.
- Bedingfield, J. B., Calder, L. D., & Karler, R. (1996). Comparative behavioral sensitization to stereotypy by direct and indirect dopamine agonists in CF-1 mice. *Psychopharmacology*, 124, 219–225.
- Bell, K., Duffy, P., & Kalivas, P. W. (2000). Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology*, 23, 335–344.
- Bourne, J. A. (2001). SCH 23390: The first selective dopamine D1-like receptor antagonist. *CNS Drug Reviews*, 7, 399–414.
- Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin*, 114, 80–99.
- Brunelli, M., Magni, F., Moruzzi, G., & Musumeci, D. (1975). Apomorphine pecking in the pigeon. *Archives Italiennes de Biologie*, 113, 303–325.
- Caine, S. B., Heinrichs, S. C., Coffin, V. L., & Koob, G. F. (1995). Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. *Brain Research*, 692, 47–56.
- Cheng, H. C., & Long, J. P. (1974). Dopaminergic nature of apomorphine-induced pecking in pigeons. *European Journal of Pharmacology*, 26, 313–320.
- Crombag, H. S., Badiani, A., Chan, J., Dell'Orco, J., Dineen, S. P., & Robinson, T. E. (2001). The ability of environmental context to facilitate psychomotor sensitization to amphetamine can be dissociated from its effect on acute drug responsiveness and on conditioned responding. *Neuropsychopharmacology*, 24, 680–690.
- Crombag, H. S., Badiani, A., Maren, S., & Robinson, T. E. (2000). The role of contextual versus discrete drug-associated cues in promoting the induction of psychomotor sensitization to intravenous amphetamine. *Behavioural Brain Research*, 116, 1–22.
- Delius, J. D. (1985). The pecking of the pigeon: Free for all. In C. F. Lowe, M. Richelle, D. E. Blackman, & C. M. Bradshaw (Eds.), *Behavior analysis and contemporary psychology* (pp. 53–81). New York: Erlbaum.
- Delius, J. D., Acerbo, M. J., Keller, S., & Godoy, A. M. (2001). Drogen-induziertes Lernen: Sensitivierung bei Apomorphin [Drug-induced learning: Sensitization to apomorphine]. *Neuroforum*, 4, 261–266.
- Delius, J. D., Krug, I., Leydel, R., Keller, S., & Acerbo, M. J. (2004). *Diverse effects of apomorphine on the behavior of pigeons*. Manuscript in preparation.
- Deviche, P. (1984). Administration of small doses of apomorphine attenuates feeding in non-deprived pigeons. *Physiology & Behavior*, 33, 581–585.
- Djamo, M. B., & Wagmer, H. J. (1992). Localization and function of dopamine in the adult vertebrate retina. *Neurochemistry International*, 20, 139–191.
- Dubbledam, J. L. (1998). Birds. In R. Nieuwenhuys, H. J. Ten Donkelaar, & C. Nicholson (Eds.), *The central nervous system of vertebrates* (pp. 1526–1636). Berlin: Springer.
- Durstewitz, D., Kröner, S., & Güntürkün, O. (1999). The dopaminergic innervation of the avian telencephalon. *Progress in Neurobiology*, 59, 161–195.
- Godoy, A. M. (2000). *Dopamine and learning in the pigeon (Columba livia)*. Doctoral thesis. University of Konstanz, Konstanz, Germany.
- Godoy, A. M., & Delius, J. D. (1999). Sensitization to apomorphine in pigeons due to conditioning, subject to generalisation but resistant to extinction. *Behavioural Pharmacology*, 10, 367–378.
- Hilditch, A., Drew, G. M., & Naylor, R. J. (1984). SCH 23390 is a very potent and selective antagonist at vascular dopamine receptors. *European Journal of Pharmacology*, 97, 333–334.
- Hinson, R. E., & Poulos, C. X. (1981). Sensitization to the behavioral effects of cocaine: Modification by Pavlovian conditioning. *Pharmacology Biochemistry and Behavior*, 15, 559–562.
- Hörster, W., Krumm, E., Mohr, C., & Delius, J. D. (2002). Conditioning the pecking motions of pigeons. *Behavioural Processes*, 58, 27–43.
- Hyttel, J. (1983). SCH 23390—the first selective dopamine D-1 antagonist. *European Journal of Pharmacology*, 91, 153–154.
- Järbe, T. U. (1984). Discriminative stimulus properties of cocaine: Effects of apomorphine, haloperidol, procaine and other drugs. *Neuropharmacology*, 23, 899–907.
- Kalant, H. (1989). Drug tolerance and sensitization: A pharmacological overview. In A. J. Goudie (Ed.), *Psychoactive drugs* (pp. 547–577). Clifton, NJ: Humana Press.
- Karten, H. J., & Hodos, W. (1967). *A stereotaxic atlas of the brain of the pigeon (Columba livia)*. Baltimore: Johns Hopkins University Press.
- Kebabian, J. W., & Calne, D. B. (1979, January 11). Multiple receptors for dopamine. *Nature*, 277, 93–96.
- Keller, S., & Delius, J. D. (2001). Discriminative learning occasioned by the administration of a dopamine agonist. *Psychopharmacology*, 157, 320–323.
- Keller, S., Delius, J. D., & Acerbo, M. J. (2002). Apomorphine sensitization: Evoking conditions, context dependence, effect persistence and conditioned nature. *Behavioural Pharmacology*, 13, 189–201.
- Kelley, A. E. (1999). Neural integrative activities of nucleus accumbens subregions in relation to learning and motivation. *Psychobiology*, 27, 198–213.
- Kostrzewa, R. M. (1995). Dopamine receptor supersensitivity. *Neuroscience & Biobehavioral Reviews*, 19, 1–17.
- Lappalainen, J., Hietala, J., Pohjalainen, T., & Syvalahti, E. (1992). Regulation of dopamine D1 receptors by chronic administration of structurally different D1 receptor antagonists: A quantitative autoradiographic study. *European Journal of Pharmacology*, 210, 195–200.
- Laudrup, P., & Wallace, L. J. (1999). Sensitization elicited by directly and indirectly acting dopaminergic agonists: Comparison using neural network analysis. *Psychopharmacology*, 141, 169–174.
- Lindenblatt, U., & Delius, J. D. (1987). Apomorphine-induced pecking in pigeons classically conditioned to environmental cues. *Psychopharmacology*, 93, 223–225.
- Lublin, H., Gerlach, J., & Peacock, L. (1993). Chronic treatment with the D1 receptor antagonist, SCH 23390, and the D2 receptor antagonist, raclopride, in cebus monkeys withdrawn from previous haloperidol treatment. Extrapyramidal syndromes and dopaminergic supersensitivity. *Psychopharmacology*, 112, 389–397.
- Machlis, L. M. (1980). Apomorphine: Effects on the timing and sequencing of pecking behavior in chicks. *Pharmacology Biochemistry and Behavior*, 13, 331–336.
- Mattingly, B. A., & Gotsick, J. E. (1989). Conditioning and experiential factors affecting the development of sensitization to apomorphine. *Behavioral Neuroscience*, 103, 1311–1317.
- Mattingly, B. A., Koch, C., Osborne, F. H., & Gotsick, J. E. (1997).

- Stimulus and response factors affecting the development of behavioral sensitization to apomorphine. *Psychopharmacology*, 130, 109–116.
- Moller, H. G., Nowak, K., & Kuschinsky, K. (1987). Conditioning of pre- and post-synaptic behavioural responses to the dopamine receptor agonist apomorphine in rats. *Psychopharmacology*, 91, 50–55.
- Motles, E., Gomez, A., Tetas, M., & Gonzales, M. (1993). Effects of SCH 23390 and sulpiride on the behaviors evoked by amphetamine and apomorphine in adult cats. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 17, 1005–1022.
- Nakano, K., Kayahara, T., Tsutsumi, T., & Ushiro, H. (2000). Neural circuits and functional organization of the striatum. *Journal of Neurology*, 247(Suppl. 5), VI–V15.
- Osuide, G., & Adejoh, P. O. (1973). Effects of apomorphine and its interactions with other drugs in the domestic fowl. *European Journal of Pharmacology*, 23, 56–66.
- Randall, P. K. (1985). Quantification of dopaminergic supersensitization using apomorphine-induced behavior in the mouse. *Life Science*, 37, 1419–1423.
- Richtand, N. M., Kelsoe, J. R., Kuczenske, R., & Segal, D. S. (1997). Quantification of dopamine D1 and D2 receptor mRNA levels associated with the development of behavioral sensitization in amphetamine treated rats. *Neurochemistry International*, 31, 131–137.
- Robbins, T. W., & Everitt, B. J. (2002). Limbic-striatal memory systems and drug addiction. *Neurobiology of Learning and Memory*, 78, 625–636.
- Schwartz, R. A., Greenwald, E. R., Fletcher, P. J., Houle, S., & DaSilva, J. N. (2003). Up-regulated dopamine D1 receptor binding can be detected in vivo following repeated SCH 23390, but not SKF 81297 or 6-hydroxydopamine, treatments. *European Journal of Pharmacology*, 459, 195–201.
- Sealfon, S. C., & Olanow, C. W. (2000). Dopamine receptors: From structure to behavior. *Trends in Neurosciences*, 23, S34–S40.
- Severson, J. A., Robinson, H. E., & Simpson, G. M. (1992). Neuroleptic-induced striatal dopamine receptor supersensitivity in mice: Relationship to dose and drug. *Psychopharmacology*, 84, 115–119.
- Siemann, M., & Delius, J. D. (1992). Apomorphine-induced behaviour in pigeons (*Columba livia*). In N. Elsner & N. R. Richter (Eds.), *Rhythmogenesis in neurons and networks* (p. 600). Stuttgart, Germany: Thieme.
- Stewart, J., & Badiani, A. (1993). Tolerance and sensitization to the behavioral effects of drugs. *Behavioural Pharmacology*, 4, 289–312.
- Stewart, J., & Vezina, P. (1991). Extinction procedures abolish conditioned stimulus control but spare sensitized responding to amphetamine. *Behavioural Pharmacology*, 2, 65–71.
- Tirelli, E. (2001). Short-term contextual sensitization and conditioned hyperkinesia produced by cocaine in suckling rats aged 4–10 days and 14–20 days. *Psychopharmacology*, 156, 42–52.
- Tirelli, E., & Heidbreder, C. (1999). Conditioning of and contextual sensitization to apomorphine-induced climbing in mice: Evidence against the habituation hypothesis. *Behavioral Neuroscience*, 113, 368–376.
- Waddington, J. L., & Daly, S. A. (1993). Regulation of unconditioned motor behaviour by D1:D2 interactions. In J. L. Waddington (Ed.), *D1:D2 dopamine receptor interactions* (pp. 51–78). London: Academic Press.
- Wickens, J. (1990). Striatal dopamine in motor activation and reward-mediated learning: Steps towards a unifying model. *Journal of Neural Transmission*, 80, 9–31.
- Willner, P., Papp, P., Chceta, S., & Muscat, R. (1992). Environmental influences on behavioural sensitization to the dopamine agonist quinpirole. *Behavioural Pharmacology*, 3, 43–50.
- Wynne, B., & Delius, J. D. (1995). Sensitization to apomorphine in pigeons: Unaffected by latent inhibition but still due to classical conditioning. *Psychopharmacology*, 119, 414–420.
- Zarrindast, M. R., Hajian-Heydari, A., & Hoscini-Nia, T. (1992). Characterization of dopamine receptors involved in apomorphine-induced pecking in pigeons. *General Pharmacology*, 23, 427–430.
- Zavala, A. R., Nazarian, A., Crawford, C. A., & McDougall, S. A. (2000). Cocaine-induced behavioral sensitization in the young rat. *Psychopharmacology*, 151, 291–298.