Sabine Keller · Juan D. Delius

Discriminative learning occasioned by the administration of a dopamine agonist

Abstract *Rationale:* The repeated administration of psychostimulants usually brings about a progressive increment of the behavioral responses that they induce. We examined to what extent this sensitization is due to an associative learning process. *Objectives:* The dopamine agonist apomorphine elicits stereotyped pecking in pigeons, a response that increases with successive intramuscular injections. We tested whether this sensitized pecking would be discriminatively directed at environmental stimuli that had been present during the sensitization phase. *Methods:* In a preliminary experiment we identified a pair of stimulus compounds that attracted an equal number of apomorphine peck responses. During discrimination training naive pigeons were exposed on 5 days to both a cage furnished with one of these stimuli after having been injected with apomorphine and to a cage furnished with the other stimuli after having been injected with saline. Then the birds were administered apomorphine (or saline) and tested in a cage that offered both compound stimuli simultaneously. A discrimination reversal training and renewed tests followed. *Results:* The tests under apomorphine and saline showed that the pecking by the pigeons was virtually exclusively aimed at the specific environmental stimuli under which the sensitization to apomorphine had taken place. This discriminative stimulus control was reversed after the pigeons had been retrained with converse stimulus compound allocations. *Conclusions:* The sensitized apomorphine pecking of pigeons was subject to close control by environmental stimuli. The results thus support the hypothesis that the sensitization to psychostimulants may be due to a conditioning process. The conditioning occasioned by apomorphine injections in birds could be a useful model for the study of sensory-motor learning processes.

Introduction

The repeated administration of many drugs yields the development of tolerance, that is, a progressive diminution of the response to the particular dose employed. In most cases this tolerance appears to result from the activation of a synaptic down-regulating process. This mechanism counteracts the excessive excitation of the pathways that ultimately trigger the behavioural effects of the relevant drug. Even so, there is evidence that in at least some cases the development of tolerance is accompanied by a conditioning to contextual stimuli (Kim et al. 1999). Psychostimulant drugs differ from most other psychoactive drugs in that they tend to yield an increasing behavioural effect upon multiple administrations of one and the same dose. Such progressive sensitization of a locomotor activity response up to a dose-dependent asymptote has been repeatedly demonstrated in mice and rats using amphetamine and cocaine, addictive substances that are both indirect and somewhat unspecific agonists of the transmitter dopamine (Stewart and Badiani 1993; Anagnostaras and Robinson 1996). Apomorphine, a nonaddictive but potent direct agonist of dopamine, also brings about sensitization of the locomotor response of rats (Mattingly et al. 1997). There is still some uncertainty about the mechanism responsible for the sensitization to these psychostimulants, some authors assuming that the sensitization is mainly due an unspecified up-regulatory synaptic mechanism triggered by the drugs' repeated administration, modulated perhaps by some non-associative learning.

There is, however, the possibility that the sensitization may also be at least partly due to an associative learning process. According to this view, the administration of the drug acts as an unconditioned stimulus (US) and the effect of the drug administration, a bout of increased behav-

S. Keller · J.D. Delius (✉)
Allgemeine Psychologie, Universität Konstanz, 78457 Konstanz, Germany
e-mail: juan.delius@uni-konstanz.de
Fax: +49-7531-883184

ioural activity, represents an unconditioned response (UR). The particular environment, often the actometer in which the effect of the drug is measured, functions as a conditioned stimulus (CS). Repeated pairings of drug administration and this special environment is assumed to lead to the development of a conditioned response (CR), an increase in activity triggered by the specific context stimuli. When the drug is repeatedly administered, the CR cumulates onto the activity UR directly elicited by the drug. This effectively yields a sensitization-like increase of the total response. However, the conditioning explanation of sensitization is challenged by the finding that the CR elicited by the CS alone – that is the response shown when the subjects are exposed to the CS context while not being drug treated – tends to fall short of the response increment shown in connection with the sensitization to the drug. Also, the magnitude of sensitization is usually negligibly affected by latent inhibition or inhibitory extinction treatments. These two CS-alone treatments instituted either before or after the drug sensitization course have typically strong response suppressing effects in more conventional conditioning preparations. The conditioning hypothesis, however, is supported by the finding that the sensitization increments are usually attenuated when the drug-challenge is shifted to a different test environment than the sensitization environment (Anagnostaras and Robinson 1996). The degree to which and the mechanism by which the contextual stimuli come to control the sensitized responses thus still remain a disputed issue. An apparently widespread opinion is that the environmental stimuli only facilitate the drug responses to a limited extent through non-associative modes of learning (Stewart and Badiani 1993; Mattingly et al. 1997). We now report results showing that an apomorphine-elicited behaviour response did come under a strongly discriminative stimulus control in an experiment that ensured an associative learning process.

The issue was investigated in a non-mammalian species that prominently displays a more specific response to apomorphine than the locomotory activity increase predawn dominantly shown by rodents. When apomorphine (between about 0.2 mg and 2 mg/kg bodyweight) is intramuscularly administered, pigeons and other birds (except occasional genetically non-responsive individuals) exhibit an about 30-min to 2-h long, dose dependant bout of repetitive pecking activity. The pecks are stereotypically and idiosyncratically directed at contrasting cage details (cross-welds, rivet heads, coloured dots) but sometimes also directed at features of their own integument (feathers, toes; Brunelli et al. 1975; Machlis 1980). Although the pecking may also be directed at grains, these are rarely swallowed since apomorphine has an anorectic side-effect (Deviche 1984). When pigeons are injected daily with medium doses of apomorphine in a particular cage, the pecking response to each drug administration typically increases over the first few administrations from several hundred pecks up to a few thousand pecks per 20-min observation session, indicating a pronounced sensitization (Delius 1985).

Materials and methods

Adult domestic pigeons (*Columba livia*) of local homing stock were used. They were housed in individual cages (40×45×35 cm) located in a well-lit (14 h daily) and ventilated animal room. The pigeons were drug naive before the experiments began. All treatments were carried out in accordance with the rules and regulations of the German animal protection law.

Because pigeons are well known to exhibit spontaneous stimulus preferences when pecking (Biederman et al. 1988), two different but about equally effective stimuli had to be identified in a preliminary experiment before addressing the stimulus control issue proper. To this end, a test cage of triangular prismatic shape was employed. The roof, floor and front were of wire mesh but the two back walls were lined with black and white chequered cardboard. Each of the 7.5 cm square black or white fields bore five to six scattered, about 1 cm-sized stick-on spots of various shapes and colours (Fig. 1a, right). Successive groups of three apomorphine-naive pigeons each were subjected to two test sessions on consecutive days. Before being individually placed into the test cage for 20 min, they were intramuscularly injected with 0.5 mg/kg apomorphine. Ten minutes into the second session, the birds were videotaped for the next 10 min. The first group of pigeons was exposed to green circles and red triangles on alternate white fields and yellow circles and light blue squares on alternate black fields. A slow-motion analysis of the videotapes revealed that the pigeons had mainly pecked the yellow circles on the black background. The shape and colour of the spots was modified, a new group of three pigeons was analogously tested and so on, six times over, until a pair of stimulus compounds had been found that attracted an about equivalent pecking response.

These two stimulus compounds were employed in the main, stimulus discrimination learning experiment that followed. The training cages of cubic shape had wire-mesh roofs, fronts and floors but their side and back walls were lined with white cardboard sprinkled with green circles (8 mm diameter) or alternatively with black cardboard sprinkled with red triangles (10 mm sides), at a density of about ten per 100 square cm in either case (Fig. 1a, left). On 5 successive days, 20 experimentally naive adult pigeons were individually placed into one of the training cages after a 0.5 mg/kg apomorphine injection and into the other training cages after a saline injection during two separate 30-min sessions. A quarter of the subjects were injected with apomorphine in the morning and placed in the green-white cages and then injected with saline in the afternoon and placed in the red-black cages. Another quarter were also injected with apomorphine in the morning but placed into the red-black cages and then injected with saline in the afternoon and placed into the green-white cages. The remaining half were analogously trained but completed their saline sessions in the morning and their apomorphine sessions in the afternoon. The videographs of the first sessions under saline and apomorphine were analysed for the number of pecks at the wall stimuli.

After two days rest, the birds were administered apomorphine and placed singly into the triangular test cage with both its end walls lined with green circles on a white ground and red triangles on a black ground chequered cardboard. After a five minutes delay, each bird was videotaped for 10 min. Following a break of several days, the five pigeons in each group that had pecked most during the latter test were exposed again to one apomorphine session and one saline session in the appropriate training cages. They were then tested again in the triangular cage after having been injected with saline instead of apomorphine. The videographs were analysed for the number of pecks directed at the green circles and the red triangles.

A week later, the same ten pigeons were trained on a reversed discrimination for another five pairs of daily sessions. The same procedure as during the original training was followed, but the five pigeons previously treated with apomorphine in the green-white cages were now treated with apomorphine in the red-black cages, while the five pigeons previously treated with apomorphine in the red-black cages were now apomorphine treated in the green-
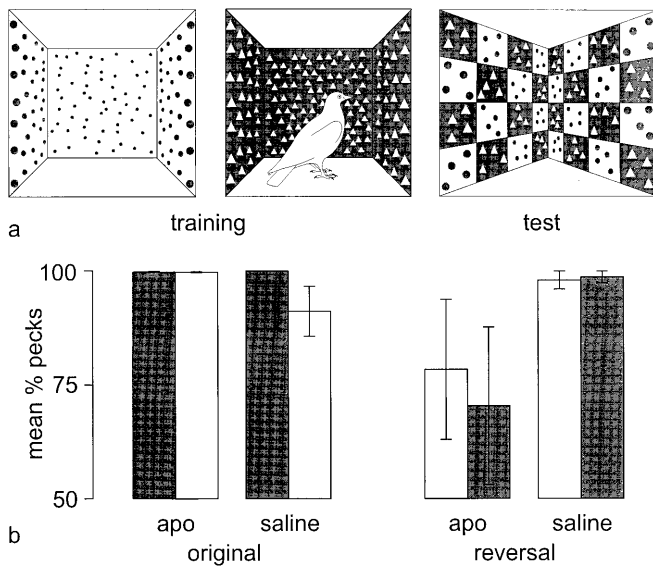
Fig. 1 a Training cages and test cage, schematic. **b** Discriminative choice of apomorphine-trained stimuli under apomorphine test and saline test conditions (means±SE percent of total number of pecks directed at apomorphine conditioned), separately shown for green circle-white background trained (*dark columns*) and red triangle-black background trained (*light columns*) pigeons, both after the original training (left) and after the reversal training (right)

white cages. The corresponding saline treatments took place in the converse cages. All pigeons were then tested as before under apomorphine in the triangular cage, exposed to an additional pair of reversal training sessions and tested again under saline. The subjects' percent of pecks directed at the apomorphine-trained stimuli out of the total number of pecks issued during the 10-min test periods were calculated.

Results

The seven birds that had not pecked the stimulus walls but instead had idiosyncratically pecked their own bodies, the cage floors, or had not pecked at all (apomorphine-unresponsive birds) were necessarily. The 13 pigeons that had in fact pecked the stimulus walls yielded a mean 430 (±477 SE) pecks per 10 min at the wall stimuli during their first apomorphine training session and 1492 (±146) such responses during their apomorphine test session, indicating that they had undergone a pronounced sensitization. All of these pigeons directed between 99.2 and 100 (average 99.8%) of their pecks at the stimuli to which they had been trained under apomorphine. Six of these birds had been drug trained in the green-white cages and seven drug-trained in the red-black cages. Because of the nearly perfect discrimination performance, there was obviously no significant difference between these two groups. When test-cage exposed after being injected with saline rather than with apomorphine they only yielded a mean 14.3 (±6.4) pecks/10 min but still aimed an average 95.6% (range 75–100) of their pecks at the apomorphine trained stimuli (Fig. 1b, left). The same pigeons had by the way issued a mean 1.7

(±2.5) pecks/10 min during the first saline training session.

When re-tested under the influence of apomorphine after the reversal training, the pigeons directed 74.4% (range 6.9–100) of their average 1802 (±143) pecks per 10 min at the newly apomorphine-trained stimuli. Still, two of the ten pigeons aimed a majority of their pecks at the originally apomorphine-trained stimuli. However, when re-tested again under saline after an additional pair of reversal training sessions all ten pigeons directed a majority of between 89.8 and 100% (average 98.3) of their mean 14.0 (±5.7) pecks at the newly apomorphine-trained stimuli.

Discussion

The results show that a few sessions of differential conditioning can bring the apomorphine-elicited pecking of pigeons under a precise discriminative control by context stimuli. Moreover, this discrimination can be reversed by appropriate reversal training. It is worth noting that with more conventional conditioning procedures comparable discrimination performances are rarely so swiftly achieved (Delius 1983). The evidence of discriminative conditioning obtained is in accordance with the previous finding that the response increment due to apomorphine sensitization is strongly attenuated when pigeons are tested in an environment markedly different from the training one but which is otherwise already familiar to them (Godoy and Delius 1999). This supports the view that the sensitization to apomorphine in pigeons is principally determined by an associative conditioning process (Lindenblatt and Delius 1987; Wynne and Delius 1995). According to these earlier studies, it is quite likely that the pecking of the pigeons that did not peck the stimulus walls in the present experiment was nevertheless also subject to a conditioned sensitization. The present experiment was simply not designed to assess a mere conditioned response enhancement but was instead aimed at measuring a conditioned discriminative responding. It is pertinent here that pigeons, like rodents, have also been shown to prefer to revisit a cage in which they were previously treated with apomorphine than to revisit one in which they were previously treated with saline (place conditioning; Burg et al. 1989). This indicates that apomorphine administration, besides eliciting a pecking response has a rewarding effect, a circumstance that is in line with much evidence that dopaminergic transmission is heavily implicated in the neural signalling of reward (Wise and Rompre 1989).

The undeniable fact that the response triggered by the context stimuli alone is of markedly smaller magnitude than the sensitization increment shown under the influence of apomorphine can be accounted for by the circumstance that besides acting as an US, apomorphine, much as other dopamine agonists, almost certainly also acts as an interoceptive CS component that is compounded with the exteroceptive CS. Such an interoceptive ef-

fect has been demonstrated in pigeons (as well as in rats) in experiments in which an apomorphine injection served as a discriminative stimulus. Conditioned to respond for food reward access, pigeons have been found to selectively peck one key when treated with apomorphine and another key when treated with saline (Jarbe 1984). Besides, it is well established that the avian (and indeed, mammalian) retinae incorporate dopaminergic synapses which are involved in determining the effective perceptions of visual stimuli (Rohrer and Stell 1995). The interoceptive CS component is obviously missing when testing takes place following a control saline treatment. For the same reason, repeated exposures to the environmental CS alone fail, in connection with apomorphine sensitization, to yield the major suppressing effects normally expected of latent inhibition and inhibitory extinction treatments (Wynne and Delius 1995; Godoy and Delius 1999).

Apomorphine administration can thus bring about strong discriminative conditioning with respect to the pecking it elicits and with respect to the environmental stimuli to which this pecking is directed. The avian apomorphine conditioning model may thus represent a useful tool for research on the dopamino-glutamatergic synaptic interactions thought to underlie most sensory-motor learning (Kelley 1999), inasmuch as the avian telencephalon very probably incorporates analogues of most, if not all of the neural components thought to be the essential substrates of such conditioning in mammals (Veenman et al. 1995; Schultz 1997; Durstewitz et al. 1998; Gargiulo et al. 1998). It may even be possible to reduce the avian model to a brain slice preparation convenient for a neurophysiological analysis.

References

Anagnostaras SG, Robinson TE (1996) Sensitization to the psychomotor stimulant effects of amphetamine. Modulation by associative learning. Behav Neurosci 110:1397–1414

Biederman GB, McDonald KG, Heighington GA, Vanayan M (1988) Color preference in pigeons: stimulus intensity and reinforcement contingency effects in the avoidance of blue stimuli. J Exp Anal Behav 49:265–273

Brunelli M, Magni F, Moruzzi G, Musumeci D (1975) Apomorphine pecking in the pigeon. Arch Ital Biol 113:303–325

Burg B, Haase C, Lindenblatt U, Delius JD (1989) Sensitization to and conditioning with apomorphine in pigeons. Pharmacol Biochem Behav 34:59–64

Delius JD (1983) Learning. In: Abs M (ed) Physiology and behaviour of the pigeon. Academic Press, London, pp 327–355

Delius JD (1985) The pecking of the pigeon: free for all. In: Lowe CF, Richelle M, Blackman DE, Bradshaw CM (eds) Behavior analysis and contemporary psychology. Erlbaum, New York, pp 53–81

Deviche P (1984) Administration of small doses of apomorphine attenuates feeding in non-deprived pigeons. Physiol Behav 33:581–585

Durstewitz D, Kröner S, Hemmings JR, Güntürkün O (1998) The dopaminergic innervation of the pigeon telencephalon: distribution of darp-32 and cooccurrence with glutamate decarboxylase and tyrosin hydroxylase. Neuroscience 83:763–769

Gargiulo PA, Siemann M, Delius JD (1998) Shape discrimination in pigeons impaired by glutaminergic blockade of nucleus accumbens. Physiol Behav 63:705–709

Godoy AM, Delius JD (1999) Sensitization to apomorphine in pigeons is due to conditioning, subject to generalisation but resistant to extinction. Behav Pharmacol 10:367–378

Jarbe TU (1984) Discriminative stimulus properties of cocaine; effects of apomorphine, haloperidol, procaine and other drugs. Neuropharmacology 23:899–907

Kelley AE (1999) Neural integrative activities of nucleus accumbens subregions in relation to learning and motivation. Psychobiology 27:198–213

Kim JA, Siegel S, Patenall VRA (1999) Drug-onset cues as signals: intraadministration associations and tolerance. J Exp Psychol Anim Behav Proc 25:491–504

Lindenblatt U, Delius JD (1987) Apomorphine-induced pecking in pigeons classically conditioned to environmental cues. Psychopharmacology 93:223–225

Machlis L (1980) Apomorphine effects on the timing and sequencing of pecking behavior in chicks. Pharmacol Biochem Behav 13:331–336

Mattingly BA, Koch C, Osborne FH, Gotsick JE (1997) Stimulus and response factors affecting the development of behavioral sensitisation to apomorphine. Psychopharmacology 130:109–116

Rohrer B, Stell WS (1995) Localisation of putative dopamine D_2-like receptors in the chick retina, using in situ hybridization and immunocytochemistry. Brain Res 695:110–116

Schultz W (1997) Dopamine neurons and their role in reward mechanisms. Curr Opin Neurobiol 7:191–197

Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4:289–312

Veenman CL, Wild JM, Reiner A (1995) Organization of the avian "corticostriatal" projection system: a retrograde and anterograde pathway tracing study in pigeons. J Comp Neurol 354:87–126

Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191–225

Wynne B, Delius JD (1995) Sensitization to apomorphine in pigeons: Unaffected by latent inhibition but still due to classical conditioning. Psychopharmacology 119:414–420