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Dose shift effects on an apomorphine-elicited response

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Abstract: Apomorphine (Apo) a dopaminergic agonist, elicited protracted bouts of pecking when injected intramuscularly into pigeons. Repeated injections of either a small or a large dose of Apo into two separate groups of pigeons led to progressive increments in pecking up to two different asymptotic response levels (dose-related sensitization). A subsequent switch of the Apo doses between the groups yielded two statistically undistinguishable asymptotic response levels. A smaller dose of Apo induced a significantly higher asymptotic response if the pigeons had been pre-treated with a larger dose than if they had not. The results are discussed in relation to a simple classical conditioning model of sensitization, and are related to behavioural contrast phenomena that occur in conventional conditioning paradigms involving changes of food reward magnitudes.

Keywords: apomorphine, dopamine, drug sensitization, dose shift, classical conditioning, behavioural contrast, pigeons

Introduction: In therapeutic practice, it is frequently necessary to modify the dose of a drug routinely administered to a patient in the course of treatment optimization. A common assumption among practitioners is that apart from some transitory deviations, a given dose of a drug will yield an effect proportionate with the dose size regardless of the dosages that were administered beforehand.

However, repeated administration of certain drugs is accompanied by the development of a persistent tolerance or a lasting sensitization. These enduring effects could affect the efficacy of different subsequent doses of the same drug. The uncertainty that surrounds this issue, especially when psychoactive drugs are involved, is not surprising in view of the lack of sufficiently documented experimental studies on dose-shift effects.

Our interest in this matter arose in connection with the important role that learning processes seem to play in the development of tolerance and sensitization to several psychoactive drugs. A decade ago Siegel [1] proposed that tolerance to morphine developed because environmental cues repeatedly accompanying its intake became associated with its effects and gradually came to elicit a compensatory reaction, which reduced the drug's effectiveness. More recently other authors have suggested that the development of the sensitization that arises with repeated administrations of psychostimulant drugs such as cocaine and amphetamine could also be partially caused by the gradual addition of a synergistic conditioned response to the environmental stimuli accompanying the drug intake [2,3].

In agreement with Pavlov [4] drugs are viewed as unconditioned stimuli (US) which elicit unconditioned responses (UR) and the environmental cues as conditioned stimuli (CS), which come to elicit conditioned responses (CR) through repeated paired CS–US presentations. In this context dose changes can be viewed as intervening changes in the US magnitude that might affect the conditioning processes. Indeed, switches of US magnitude in more conventional conditioning contexts yield non-trivial response level alterations known as behavioural contrast effects [5,6].

Apomorphine (Apo), a potent, direct dopaminergic agonist, is used clinically as an emetic but also as an anti-parkinson drug [7]. When administered to pigeons, it elicits a persistent bout of repetitive stereotyped pecking. These pecks, mostly directed at small contrasting visual features, are motorically similar to foraging pecks [8] but since Apo [9] has an anorexic effect, they rarely lead to food ingestion. Apo-induced pecking can be conditioned to visual surrounding cues with a Pavlovian differentiation paradigm [10]. The pecking effects elicited by Apo acts as a US, eliciting unconditioned pecking (UR).

After repeated Apo injections within the same experimental cage, which acts as a CS, conditioned pecking (CR) occurs in response to that particular cage even in the absence of Apo. With repeated injections of the same Apo dose the pecking response gradually increases or sensitizes up to a dose-dependent asymptotic level [11,12]. This sensitization, which has a half-life of about 2 years [13], is apparent in the Apo training cage but not in a control saline training cage [14,15].

We have published experimental evidence showing that this environment-dependent sensitization is exclusively due to the addition of the developing CR pecking elicited by the cage CS to the UR pecking elicited by the Apo US [16]. Earlier studies on the sensitization of Apo-induced locomotory responses in rats had not been able to discount wholly the intervention of other, non-conditioning processes [17,18].

Here we assess whether shifts between a smaller and a larger Apo dose can induce behavioural contrast-like effects influencing the asymptotic responses to the doses, or whether the responses to each dose settle down to the same levels regardless of the previous dosage histories.

Materials and methods: The experimental subjects were eight adult, experimentally naive domestic pigeons (*Columba livia*) weighing between 450 and 550 g, of local homing stock, bred at the university's vivarium. They were housed in individual cages (40 × 40 × 40 cm) located in a well-ventilated, brightly lit room with a 12:12 h light/dark cycle with *ad libitum* access to water and mixed grain. All treatments complied with the German animal protection laws and regulations.

A distinctive experimental cage with its back- and side-walls lined with white paper panels sprinkled with dark-green dots (0.8 mm in diameter, about 10 dots per 100 cm^2) located in a separate room served as the environmental CS. The experiment comprised two consecutive phases of six daily sessions each. For each session pigeons were intramuscularly injected with Apo and immediately placed into the experimental cage for 20 min where the pigeons' behaviour was video-recorded. The videotapes were later reviewed and the number of pecks emitted by each pigeon in each session was recorded.

Two different Apo doses were used: a smaller (s) dose of 0.2 mg/kg and a larger (L) dose of 1.0 mg/kg. Pigeons were randomly allocated to two groups of four animals each. Group sL received the s dose during the first phase and the L dose during the second phase. Group Ls received the Apo doses in the inverse order. Mean total pecks per session (p/s) and SEMs were computed for each group. The asymptotic responses of each group at the end of the first or the second phase were estimated by pooling the corresponding means of sessions of 5 and 6 or 11 and 12.

Statistical analyses were carried out using permutation tests for intra- and inter-group comparisons [19]. These tests directly yield probability estimated P without any intermediate statistics. Any P value < 0.05 was considered significant.

Results: Figure 1b presents the recorded mean per-session responses. One pigeon of group sL had to be excluded from the computations because if never pecked in response to Apo. Apo-unresponsive individuals do occasionally occur in our pigeon stock. As expected, during the first phase the responses of both groups increased and reached clearly different dose-dependent asymptotes (sessions 5/6, sL < Ls, $P < 0.05$). During the second phase, the response of group sL with the L dose increased up to a new asymptote. This was lower (though not significantly) than the asymptote of group Ls in the first phase also with the L dose.

The response of group Ls with the s dose during the second phase decreased to a new lower asymptote, which was significantly higher than the asymptote of group SL in the first phase also with the s dose (sessions 11/12, Ls > sessions 5/6,sL, $P < 0.05$. The asymptotic responses of groups sL and Ls at the end of the second phase did not differ significantly.

During the first phase both groups displayed the expected dose-dependent response sensitization. The group injected with the L dose reached a significantly higher asymptote than the group injected with the s dose. During the second phase the responses of both groups reached new asymptotes which did not differ significantly. The experience with either Apo dose during the first phase seems to have generally reduced the asymptote dose dependency during the second phase.

Pre-treatment with the L dose definitely enhanced the subsequent asymptotic response to the s dose while pre-treatment with the s dose appears to have slightly reduced the subsequent asymptotic response to the L dose. This is equivalent to a clear-cut inverse negative, and a less pronounced inverse positive contrast effect, respectively.

As already explained, the recorded total pecking response to any Apo administration can be considered as a Cr + UR. Approximate UR estimates for reach Apo dose can be derived from the response during the first 5 min after the first Apo administration that is, before any appreciable conditioning could possibly have taken place.

The averaged responses during the first 5 min were 71 pecks for the s dose and 314 pecks for the L dose. Thus the estimated URs elicited by each dose during a 20 min session were 284 p/s and 1256 p/s respectively. Figure 1a was constructed by subtracting the URs from the matching total response scores. It shows that the estimated CR of group sL in sessions 11/12 was somewhat lower than the CR of group Ls in sessions 5/6 while the CR of group Ls in sessions 11/12 was markedly higher than the CR of group sL in sessions 5/6. This also corresponds to a sizeable inverse negative and a small inverse positive behavioural contrast effect.

The conditioning induced by each Apo dose was also plotted as the proportions between the estimated CR and the corresponding UR scores in Figure 1c. On this relative basis, the s dose yielded more conditioning than the L dose during the first phase. The levels of the CR/UR scores after the Apo dose switch suggested sizeable positive and small negative contrast effects.

The proportion of the Apo concentrations in the s (0.2 mg.kg) and the L (1.0 mg/kg) doses was 1/5 while the proportion of their corresponding estimated URs, 284 and 1256 p/s respectively, was approximately 1/4. This suggests an already slightly reduced efficacy of the L dose in triggering the pecking UR.

Discussion: In conditioning, on an absolute basis a small US generally triggers a small UR and, in conjunction with a given CS, it generates a small CR. In turn, a large US tends to trigger a large UR and to generate a large CR in response to the same CS [5].

The s dose was estimated to yield an UR of 284 p/s and the asymptotic recorded response CR + UR of group sL at the end of the first phase was 1623 p/s. Thus the estimated final CR was 1623 – 284 = 1339 p/s. Similarly, the L dose yielded an estimated UR of 1256 p/s and the asymptotic response of group Ls at the end of the first phase was 3801 p/s. Consequently, the estimated final CR was 3801–1256 = 2545 p/s (Figures 1a, 1b).

Immediately after the dose switch, group Ls could be expected to produce a UR + CR response of 284 + 2545 = 2829 p/s, which reasonably agrees with the actual 2853 p/s recorded in session 7 (Figure 1b). Similarly, group sL could be predicted to produce a post-switch Ur + CR response equal to 1256 + 1339 = 2595 p/s, which clearly exceeds the 2070 p/s recorded in session 7 (Figure 1b). Obviously the first phase CR transferred only partially over into the second phase.

A possible explanation is that the CR due to the US is more rapidly forgotten than the CR due to the US. The post-switch response of group sL further sensitized to an asymptote of 3006 p/s (Figure 1b), which was lower (though not significantly) than the expected UR + CR of 3801 p/s.

It could be argued that the CS might have lost efficacy due to familiarization during the first phase and consequently produced a smaller CR during the second phase (Figure 1a). Other studies, however, indicate that pigeon Apo conditioning is remarkably resistant to inhibitory-type treatments [14,16].

The switch from US to US experienced by group Ls led to a slight response reduction down to an asymptote of

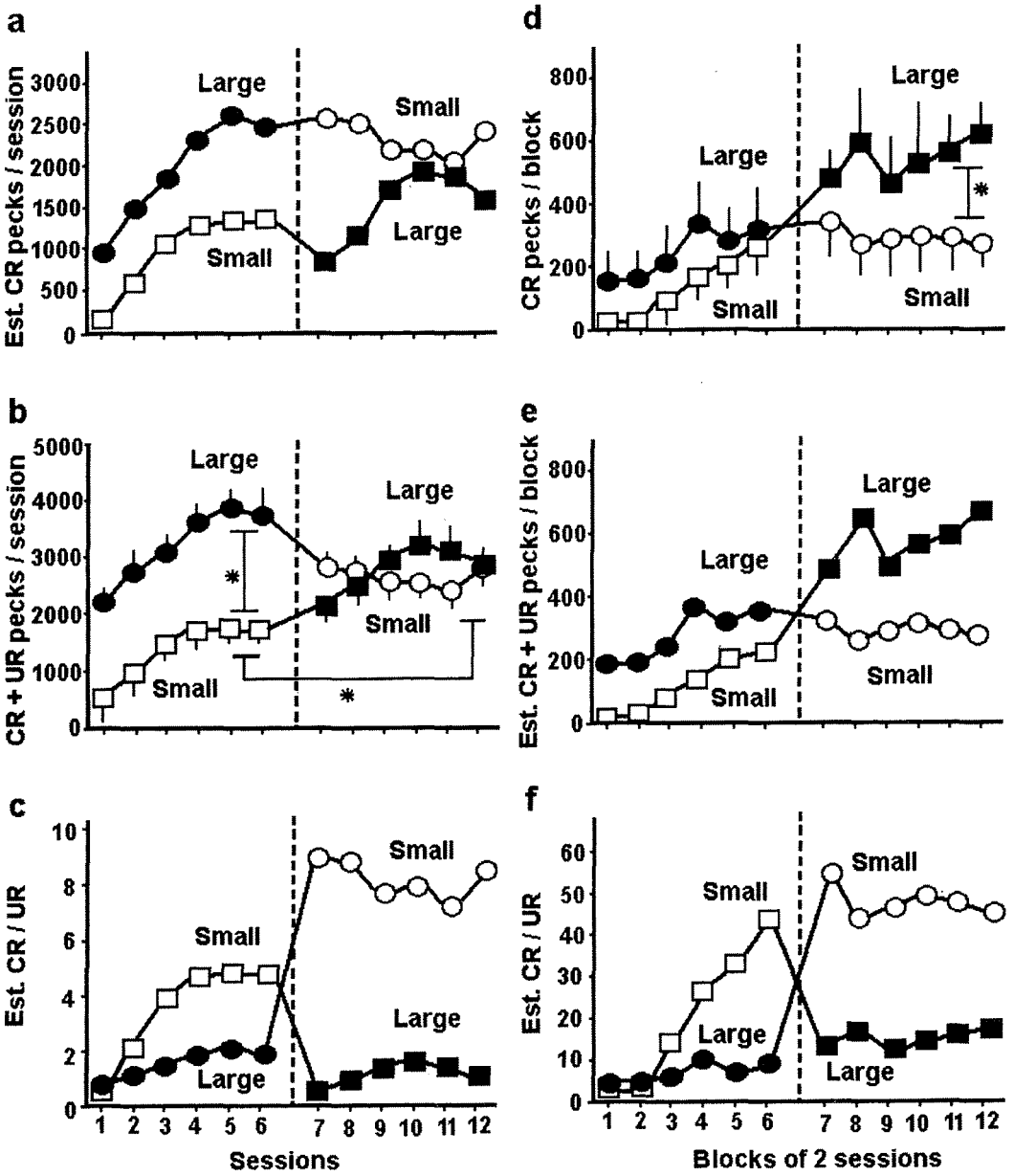

Figure 1. Left: Apo experiment: (a) estimated CR pecks per session; (b) averaged CR plus UR pecks per session (with SEM; original data); and (c) estimated CR/UR proportion. Filled symbols correspond to the larger (L) Apo dose, empty symbols to the smaller (s) Apo dose Right: Food experiment briefly mentioned in the discussion. (e) Averaged CR pecks per session (with SEM; original data). (f) Estimated CR plus UR pecks per session. (g) CR/UR proportion. Filled symbols correspond to the larger (L) food US, empty symbols to the smaller (s) food US. In all panels the sL group is identified by squares and the Ls group by circles. The starred end-stopped lines refer to statistically significant differences mentioned in the text.

2533 p/s (Figure 1b), which was significantly higher than the expected CR + UR of 1623 p/s. Thus the CR was apparently highly persistent, which agreed with earlier findings [15], while the reduced US was obviously not particularly effective in inhibiting it (Figure 1a).

The CR/UR proportions may be more adequate than the absolute response scores to compare the conditioning induced by different Apo doses. Figure 1c shows that the smaller US initially evoked stronger relative CR than the larger US. This outcome is not unusual since ceiling effects resulting from a too intense UR can curtail the development of the CR [5]. Actually, the first asymptotic CR + UR of 3801 p/s elicited by the L dose (Figure 1b) exceeded the maximal response rate of about 3 pecks/second that pigeons ca normally maintain [11] and probably leaves no room for a potentially stronger CR.

Additionally, the L dose might have been relatively less effective than the s dose because of incipient coordination impairment and retching responses, that are quite obvious with larger Apo doses (e.g. 10 mg/kg; unpublished observations). In any case, the CR/UR proportions fit in well with the mechanics of some current mathematical models of conditioning [5]. Such a modelling of Apo dose switch results may indeed be worthwhile once more data are available.

To assess whether the Apo dose shift effects could be related to behavioural contrast, we ran a subsidiary experiment. Eight mildly food-deprived pigeons learned to associate food delivery (US) from an overhead solenoid feeder with a visual stimulus (CS) displayed under a transparent pecking key with a light-emitting diode matrix. The conditioning device [20] was attached to the pigeons' home cages.

The experiment consisted of daily sessions of 40 trials each, every trial starting with a 20 s interval, followed by an 8 s CS presentation and ending with food delivery. The CR pecks on the key were recorded during the CS presentations but the food-directed UR pecks were too weak to activate the key and had to be estimated from the number of grains delivered [21]. During the first phase group sL received a US of about three millet grains and group Ls a US of about 18 millet grains. For the second phase the US food rations were switched between groups.

The results are plotted in 12 blocks of two sessions in Figures 1d (CRs), 1e (CRs + URs) and 1f (CR/UR). Both the CR + UR data of group Ls exhibited no negative behavioural contrast while those of sL revealed an almost significant positive contrast (blocks 5/6, Ls < blocks 11/12, sL, $P < 0.06$). Thus, the response pattern differed from that obtained in the Apo experiment (Figures 1a, 1b). However, more similar effects to those accompanying the Apo dose switch have been observed in other food-conditioning studies [22]. Note though that the CR/UR ratio plots of our two experiments (Figures 1c, 1f) exhibit considerable similarity.

A full explanation for the effects of Apo dose changes requires further experiments examining whether repeated switches between the two different Apo doses lead to stable response levels or whether pre-treatments with a smaller or a larger Apo dose leads to different response levels to a subsequent intermediate dose. But regardless of the results of such experiments, it is certain that an animal's response to a particular Apo dose can be significantly influenced by its previous experience with another Apo dose.

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