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ORIGINAL INVESTIGATION

# Nicotine withdrawal and reward responsivity in a card-sorting task

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**Abstract**
*Rationale* Animal studies have demonstrated decreased reward responsivity during nicotine withdrawal (e.g., Epping-Jordan et al., Nature 393:76–79, 1998) and the Card Arranging Reward Responsivity Objective Test (CARROT) has recently been used to study the effect of nicotine withdrawal on reward responsivity in humans (e.g., Al-Adawi and Powell, Addiction 92:1773–1782, 1997; Powell et al., Biol Psychiatry 51:151–163, 2002). We investigated a suggestion that nicotine withdrawal may have additional reward-related effects apart from the reward responsivity effects already observed.
*Objective* The objective of this study was to determine whether or not nicotine withdrawal results in slower improvements in performance on a card-sorting task over a series of trials.
*Method* We carried out two experiments using a modified version of the CARROT, the mCARROT, to compare the performance of human participants in nicotine withdrawal with those who were satiated.
*Results* Although withdrawal produced no direct effect on the mCARROT measure of reward responsivity, the overall sorting rate was lower, and the increase in sorting rate across successive trials was slower during nicotine withdrawal than during satiation.
*Conclusions* These data indicate that nicotine withdrawal impacted on task performance independently of the introduction of a performance contingent reward, suggesting a novel reward-related effect of nicotine withdrawal.

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## Introduction

Nicotine is widely accepted as the primary component of tobacco smoke that leads to addiction (Stolerman and Jarvis 1995), but the precise details of nicotine's reinforcing action remain unclear. Two "direct reinforcement" models have been advanced to explain the development and maintenance of addiction. Positive reinforcement models propose that nicotine unconditionally reinforces the behaviors upon which it is contingent. In contrast, negative reinforcement models suggest that nicotine's reinforcing effects depend upon a pre-existing negative state such as withdrawal (for reviews, see Eissenberg 2004; Glautier 2004). Recently, however, a third model has been proposed (e.g., Epping-Jordan et al. 1998), suggesting that nicotine's effects are indirect, modifying the impact of other reinforcers rather than acting as a reinforcer in its own right. In support of this reward responsivity account, Epping-Jordan et al. (1998) demonstrated that rats undergoing nicotine withdrawal had higher thresholds for electrical brain stimulation reward. Because the site of electrical brain stimulation reward is part of a general reward system, it does not seem unreasonable to conclude that nicotine may impact other classes of reward. Although the detailed workings of a model of the biological basis of the effect of nicotine withdrawal on reward responsivity are not yet fully established, reduced dopamine function is a leading candidate mechanism (Hughes 2007).

In humans, there are a number of procedures available for studying reward. For example Glautier et al. (1998) used an operant task based on the application of

Herrnstein's (1979) Matching Law to examine the effects of ethanol and mood on sensitivity to reward. Other examples include a choice task used in a study of the role 5-HT in reward and an associative learning task used to study the effects of nicotine (Barr et al. 2008; Cools et al. 2005). A detailed study of the effect of nicotine withdrawal on reward responsivity has also been undertaken by using the Card Arranging Reward Responsivity Objective Test (CARROT; Powell et al. 1996). Al-Adawi and Powell (1997) used the CARROT to test whether nicotine withdrawal in humans produced a reduction in reward responsivity, analogous to that seen in rats using electrical brain stimulation reward. During the CARROT procedure, participants were required to sort cards under reward conditions, when a small amount of money was earned for each card sorted, and under conditions of no reward. Reward typically increased sorting speed and the magnitude of the increase constituted a measure of reward responsivity. Importantly, Al-Adawi and Powell found that reward responsivity decreased during withdrawal, and these findings were later replicated (Powell et al. 2002). Similar results were obtained by Dawkins et al. (2006) when withdrawal effects were abolished using nicotine lozenges, rather than smoked cigarettes, seemingly isolating the pharmacology of the effect more directly to nicotine itself. Thus, it seems entirely appropriate to consider altered reward function as one of the symptoms of nicotine withdrawal, among those more traditionally established, such as anxiety, difficulty concentrating, craving, and increased appetite (e.g., Hughes 1992; Pomerleau et al. 2000).

Our initial investigation (unpublished) was designed to extend these studies of nicotine withdrawal on reward and examine whether or not any effects were related to level of dependence, as measured with a widely used self-report tool, the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al. 1991). In this way, we hoped to develop some understanding of changes in reward function during nicotine withdrawal, focusing on the transition between early and later stages of dependence. If nicotine withdrawal affects reward function in the early stages of dependence, it may suggest one mechanism which differentiates those who experiment with smoking and then stop, those who experiment and become regular smokers, and those who go on to become very heavy and dependent smokers (e.g., for a discussion of transitions between initial smoking and dependence, see Eissenberg and Balster 2000). However, our initial investigations were complicated by (a) difficulties in showing reliable increases in sorting speed on rewarded trials and (b) increases in sorting rates that occurred over the first few trials of the card-sorting task whether or not reward was introduced.

In Experiment 1, therefore, we describe a *modified* CARROT task that included longer trials, was more difficult for participants to carry out, so produced slower sorting rates, and included a penalty for incorrect sorting. The mCARROT produces a reliable effect of reward and, in Experiment 1, the mCARROT was used with two rewarded and two nonrewarded trials counterbalanced for order of presentation. This meant that rewarded and nonrewarded trials occurred equally often in each position of a four-trial sequence so that any change in performance across the trial sequence affected rewarded and nonrewarded trials equally. Although this counterbalancing of order was a departure from the method used in previously published studies, it was an important step considering the changes in sorting speed across trials that we had previously observed. It also allowed for constructive replication of earlier findings.

In published studies (Al-Adawi and Powell 1997; Powell et al. 2002), the CARROT consisted of a sequence of three trials, only the second of which was rewarded. The reward responsivity measure was the difference between sorting rate in the second (rewarded) trial and the mean sorting rate obtained from the first and third (nonrewarded) trials (NRN sequence). These experiments included a single warm-up trial before the test trial series, but it remains possible that differences in pattern of change across the test trial series during nicotine withdrawal could spuriously indicate effects of withdrawal on reward responsivity. For example, if sorting began at a low rate on the first trial, reached its maximum value on the second (rewarded) trial, and remained at that asymptote on the third trial, then a comparison of sorting rate on the second trial with the mean rate on the first and third trials would give a false indication of reward responsivity compared to a condition where the increase between trials 1 and 2 was that same as that between trials 2 and 3. In fact, because nicotine withdrawal might affect reward responsivity alone, the rate of improvement following practice alone or both factors, a control for practice is an essential requirement for an unambiguous test of the reward responsivity effect.

## Experiment 1

### Participants

Thirty-two participants (five males) with a mean (*M*) age of 24.4 years (standard deviation [SD]=6.5) responded to advertisements posted on the University of Southampton campus and online. The advertisements invited self-identified smokers to apply to take part in a study on "the experience of reward in nicotine dependence", no limitations on the basis of smoking levels were stated. All participants were paid the money they earned in the

mCARROT and course credit was given to students on psychology units.

## Design and procedure

Prior to data collection, participants completed the FTND. Each participant attended the laboratory twice, once after they had abstained from smoking overnight (withdrawal condition) and once after having smoked as usual (satiation condition). To maximize the difference between conditions, participants in the satiation condition were asked to smoke a cigarette immediately before coming to the laboratory. Participants were randomly allocated to attend in one of the two possible orders of testing; half were tested in withdrawal first and half were tested in satiation first.

Upon arrival at the laboratory, participants were given an information sheet and signed a consent form. Their smoking status was verified by analysis of expired carbon monoxide (ECO) levels using a CO monitor (Bedford EC50 Micro III Smokerlyzer, Bedford Scientific Instruments, Kent, UK). Satiation was verified by ECO levels >15 parts per million (ppm) whereas overnight abstinence was verified by ECO levels ≤15 ppm. After ECO testing, participants completed the mCARROT. The mCARROT was carried out over a series of trials with each trial being carried out under specified conditions for a defined time period. The modifications to the procedures described in previous publications were as follows. First, as before, each card had five digits printed on it, of which, one and only one was either a 1, a 2, or a 3. Participants had to sort the card stack, as quickly as possible, into three correspondingly numbered boxes fixed to the desk in front of them. The boxes were introduced in the mCARROT to reduce between-participant variability in the way the cards were sorted and made the task more difficult as participants were required to make more precise movements to place each card. Second, participants were also instructed that, on rewarded trials, ten pence would be earned for every five cards correctly sorted and, for every mistake they made (including cards missing the box), ten pence would be deducted from their earnings. After an initial 2-min practice trial, the test trial series began. In the present study, the procedure also differed from the usual NRN sequence of three short (1–2 min) trials (Al-Adawi and Powell 1997; Powell et al. 2002, 1996). Instead, the test series consisted of four 5 min trials, two of which were rewarded (R) and two nonrewarded (N). Eight participants were randomly allocated to one of four trial orders (RNNR; NRRN; RNRN; NRNR).

The whole procedure lasted approximately 40 min. A debriefing statement was made available to each participant on completion. The procedure was approved by the Ethics Committee of the School of Psychology at the University of Southampton.

## Analyses

Three analyses, each comparing mCARROT performance in the withdrawal and satiation conditions, were conducted. Analyses of variance (ANOVAs) and *t* tests were used both to look at overall effects and to follow-up significant effects from the main analyses. Appropriately corrected degrees of freedom were used wherever violations of sphericity were found on repeated-measures data (Greenhouse and Geisser 1959).

## Results

A paired samples *t* test confirmed that participants differed in ECO levels in withdrawal and satiation ($t(31)=8.33$, $p<0.001$). In withdrawal, the mean ECO level was 4.8 ppm (SD=4.0) whereas in satiation it was 19.3 ppm (SD=12.7). The average FTND score was 3.5 (SD=2.33).

In the first main analysis, a 2×2×2×4 mixed-measures ANOVA was carried out using sorting rate as the dependent variable. Sorting rate was expressed as the number of cards sorted per second. The within-subject factors were status (withdrawn or satiated) and reward (nonrewarded or rewarded trials with sorting rate averaged over the two trials of each type). Although not of specific interest, the between-subjects factors of order (withdrawal test first or satiation test first and trial sequence RNNR, NRRN, RNRN, and NRNR) were included. Figure 1 shows the mean sorting rates on rewarded and nonrewarded trials under conditions of withdrawal and satiation. ANOVA indicated a significant effect of reward ($F(1,24)=19.1$, $p<0.001$), of status ($F(1,24)=5.85$, $p<0.05$), and a reward by status interaction ($F(1,24)=5.12$, $p<0.05$). Thus, reward increased sorting rate, withdrawal decreased sorting rate, and there was a strong trend toward a larger effect of reward under withdrawal, but this was opposite in direction to that expected. None of the other effects were significant with the exception of a status by order interaction ($F(1,24)=8.80$, $p<0.01$). Following up these interactions with *t* tests, the effect of status was significant in the participants who were tested under withdrawal first but not among those who were tested in satiation first ($ts(15)=3.65$ and 0.36; $ps<0.01$ and 0.73, respectively); the effect of reward was significant in both status conditions (satiation and withdrawal $ts(31)=2.56$ and 4.68; $ps<0.05$ and <0.001, respectively).

In the second analysis, the difference between the sorting rate on the second trial of the series and the average of the first and third trials was examined. A *t* test was used to compare this measure under conditions of satiation and withdrawal. This test provided a direct comparison with the

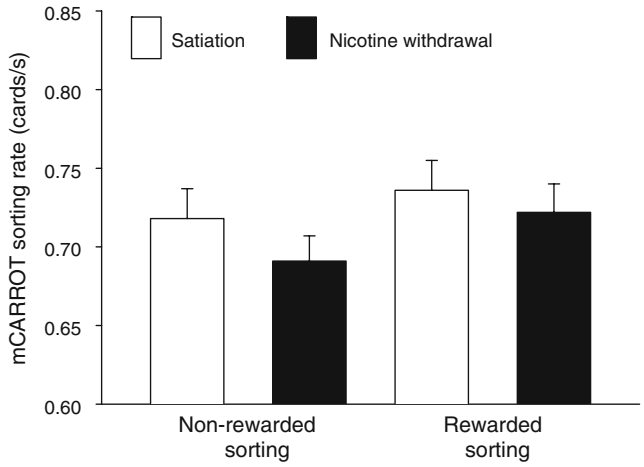


**Fig. 1** Mean (±SE) sorting rates in Experiment 1 on rewarded and nonrewarded trials under conditions of withdrawal and satiation. The total duration of the sorting task for each trial type (rewarded and nonrewarded) was 10 min. During rewarded sorting, ten pence (0.1 GBP) was earned for every five cards correctly sorted. Participants in the withdrawal condition had abstained from smoking overnight; participants in the satiation condition were asked to smoke normally and smoke a cigarette immediately before attending for the experiment. Standard error bars based on SPSS Estimated Marginal Means subcommand

analysis of Powell et al. (2002) but in the absence of any differential reward on the second trial. This derived measure was higher under satiation than under withdrawal ($M$=0.011, SD=0.026 and $M$=−0.003, SD=0.038, respectively) and a $t$ test showed this difference to be reliable ($t(31)=2.19$, $p<0.05$).

The final analysis sought to establish the pattern of sorting rates across the first three trials of the series that produced the effect of withdrawal on the derived measure. A 2×3 repeated-measures ANOVA was employed with factors status (withdrawn or sated) and trial (trials 1, 2, and 3). Figure 2 shows the mean sorting rates across these trials under conditions of withdrawal and satiation. The 2×3 ANOVA carried out on these data showed a significant effect of trial ($F(2,62)=7.69$, $p<0.01$) but neither the effect of status nor the trial by status interaction was significant ($F(1,31)=2.64$, $p=0.12$ and $F(2,62)=1.62$, $p=0.21$, respectively). However, there was a significant trial by status interaction when the quadratic trend across trials was considered ($F(1,31)=4.78$, $p<0.05$). This interaction on the quadratic trend indicated a specific pattern of difference over trials between the withdrawal and satiation conditions and was followed up with pairwise Student's $t$ tests. Although the sorting rate showed a significant increase between trial 1 and trial 3 under withdrawal ($t(31)=2.3$, $p<0.05$), neither the change between trial 1 and trial 2 nor that between trial 2 and trial 3 was significant ($ts(31)<1.8$, $ps>0.08$). In contrast, under satiation, there was a significant increase both between trials 1 and 3 ($t(31)=4.3$, $p<0.001$) and between trials 1 and 2 ($t(31)=3.5$, $p<0.01$) but the change between trials 2 and 3 was not significant ($t(31)=0.49$, $p=0.63$). Finally, comparisons of sorting rates in withdrawal and satiation at each trial showed a significant difference at trial 2 ($t(31)=2.7$, $p<0.05$) but not at trial 1 or trial 3 ($ts(31)<1.2$, $ps>0.25$).

## Discussion

The two different ways of analysing these data present something of a paradox. On the one hand, when comparing sorting rate in trial 2 with the average of trials 1 and 3, they apparently replicate a result that has been reported previously and interpreted as an effect of nicotine withdrawal on reward responsivity (Al-Adawi and Powell 1997; Powell et al. 2002). On the other hand, they not only fail to replicate the effect of nicotine withdrawal on reward responsivity but produced an effect in the opposite direction to that expected. Here, in an effort to come to an understanding of this paradox, we consider our participants, our task, and the nature of the effect of nicotine withdrawal.

The comparison of sorting rates in rewarded and nonrewarded conditions across the four trials of the mCARROT failed to produce a reduction in reward responsivity in nicotine withdrawal. This finding was unlikely to have arisen from lower levels of dependence among our participants, and hence, withdrawal severity in our sample than in samples used in previous studies. Although the FTND does not contain

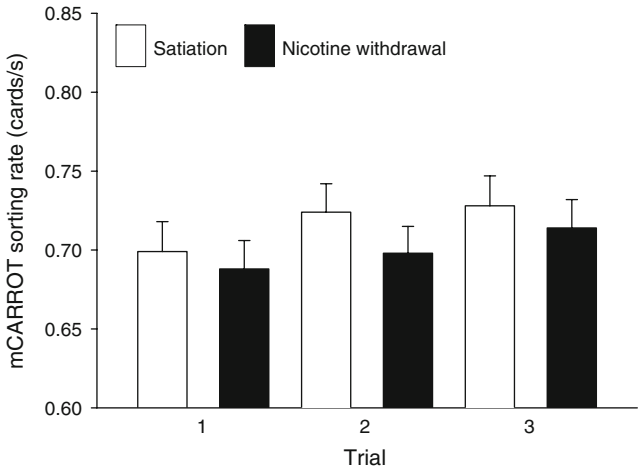


**Fig. 2** Mean (±SE) sorting rates in Experiment 1 over the first three trials under conditions of withdrawal and satiation. Each trial lasted 5 min and the data point for each trial is averaged over equal numbers of rewarded and nonrewarded conditions. During rewarded sorting, ten pence (0.1 GBP) was earned for every five cards correctly sorted. Participants in the withdrawal condition had abstained from smoking overnight; participants in the satiation condition were asked to smoke normally and smoke a cigarette immediately before attending for the experiment. Standard error bars based on SPSS Estimated Marginal Means subcommand

questions to directly assess withdrawal, scores on this questionnaire (and the earlier version, the Fagerstrom Tolerance Questionnaire; Fagerström and Schneider 1989) are positively correlated with withdrawal symptomatology (Pomerleau et al. 2000; Rios-Bedoya et al. 2008). The mean FTND score for our participants was 3.5, comparable with the smokers of Powell et al. (2002) (mean FTNDs of 3.7) and with the RAMQUIT group (mean FTND 3.2) of Al-Adawi and Powell (1997). The RAMQUIT group was tested in the CARROT procedure after stopping smoking for the entire Ramadan period. On the other hand, the DAYQUIT group of Al-Adawi and Powell (1997) had a much higher FTND ($M$= 7.9). These participants had not stopped smoking for the whole of Ramadan, instead they had just quit during the daytime over this period. Presumably, this difference in behavior was related to the different levels of dependence with more dependent participants less willing or less able to abstain for longer periods. However, DAYQUIT performance in the CARROT was comparable with the RAMQUIT group. It is of course possible that the age, gender make-up, or some other characteristic of our sample was responsible for the fact that we did not find a direct effect of withdrawal on response to reward. Our sample was younger and included fewer males than previous studies but we do not have further demographic data with which to make other comparisons.

Turning next to the task, one possible reason for the difference in the results of our study and those previously reported lies in the fact that our study counterbalanced the presentation of rewarded and nonrewarded trials so that each occurred equally often at each serial position. We have focussed our attention on this factor but we briefly consider other differences between the CARROT and mCARROT which may have also played a part—longer trials, a more difficult task, and penalties for errors. In previous versions of the task, each participant first sorted 60 cards and the time taken to do this was used in the CARROT proper as the limited period within which participants had to sort as many cards as possible. In Powell et al. (2002), the average sorting rate was 1.3 cards per second leading to an estimate of 78 s to sort 60 cards although the actual time allowed varied from participant to participant. In contrast, our trials lasted 300 s (5 min) for all participants. In addition, our task was made more difficult by requiring that sorted cards were placed accurately in a target box and warning of a penalty for incorrectly sorted cards. These effects are manifest in an overall lowering of sorting rates to between 0.6 and 0.8 cards per second. These changes were introduced after pilot studies had shown no effect of reward on sorting rate and we wished to bring rates down in the nonrewarded trials to minimize the chances of ceiling effects. Clearly, we could not look for a reduction in the effect of reward if no effect of reward was present to begin with. With these changes, the mCARROT procedure produced reliable effects of reward so should have been well placed to detect the effect of any experimental treatment that reduced reward responsivity. It is possible that the reward effects were too robust in the new procedure but it seems somewhat unlikely that these changes would have reversed the expected direction of the effect, as we seem to have observed in Experiment 1. Therefore, this was re-examined in Experiment 2.

However, as shown in Experiment 1, withdrawal was not without effects on mCARROT performance. First, it reduced overall sorting rates regardless of other factors. Second, when we examined our data over the first three trials using a derived measure based on Powell et al. (2002), we observed a result similar to that reported by Powell et al., namely, a significantly higher score on the derived measure was seen under satiation than under withdrawal. Critically, this occurred despite the fact that the counterbalancing procedure rendered each trial used to construct the derived measure identical with respect to reward and nonreward. Our trial-by-trial analysis of sorting rates showed that the effect of withdrawal was to reduce the rate at which participants approached their asymptotic sorting rate across the trial series. Sorting rates increased across trials but they did so most quickly between trials 1 and 2 under satiation. In addition, withdrawal also produced an overall reduction in sorting rate, although this effect was subject to an interaction with testing order; only participants tested in withdrawal first showed a lower sorting rate during withdrawal compared to satiation.

## Experiment 2

Powell (personal communication) kindly provided the trial-by-trial data that was used to construct the derived measure of reward responsivity reported in their 2002 paper (Powell et al. 2002). Because this data set showed similar patterns across trials to those we observed in Experiment 1, we pursued the idea that one effect of nicotine withdrawal on the mCARROT could be a reduction in the rate of increase in sorting speed across trials. To assess this, we reverted to a three-trial NRN version of the mCARROT to more closely replicate the analysis of Powell et al. (2002) but we additionally included a control group that simply received a sequence of three nonrewarded mCARROT trials (NNN). This design also allowed us to determine whether or not the surprisingly larger effect of reward in withdrawal seen in Experiment 1 based on the absolute response rates would replicate. Differences from Experiment 1 are noted below.

### Participants

The 44 participants (20 males) had a mean age of 23.2 years (SD=5.3). None had participated in Experiment 1.

## Design and procedure

Participants attended the laboratory once, either after abstaining from smoking overnight or after smoking as usual. Subject to the constraint of equal group allocation, members of both groups were randomly allocated to one of two conditions defined by the sequence of mCARROT trials (NRN or NNN). After a warm-up trial, both groups had three trials of the mCARROT. Group NRN was rewarded on the second trial whereas group NNN received three nonrewarded trials.

## Results

As in Experiment 1, analysis of ECO levels confirmed the effectiveness of the withdrawal manipulation. In withdrawal, the mean ECO level was 4.6 ppm (SD=3.2), whereas in satiation, the mean was 17.9 ppm (SD=9.7); this difference was significant ($t(43)=6.1$, $p<0.001$). The average FTND score was 2.5 (SD=2.10).

In the first main analysis, sorting rates were analyzed beginning with a 2 (status: satiated or withdrawn)×2 (group: NNN or NRN) between-subjects ANOVA carried out on the difference between the second trial and the average of the first and third. This analysis replicated that of Powell et al. (2002) but included a nonrewarded control group. The analysis showed a significant effect of group and a near significant effect of status, but no group by status interaction ($F(1,40)=13.6$, $p<0.01$; $F(1,40)=3.53$, $p=0.068$; $F(1,40)<1$, respectively). The effect of group showed that the derived measure was larger for group NRN than NNN ($M=0.024$ and $M=-0.003$, respectively), indicating an effect of reward. The near significant effect of status indicated that the derived measure was larger in satiation than in withdrawal ($M=0.018$ and $M=0.004$, respectively). However, in the complete absence of a group by status interaction, we conclude that the effect of status was equivalent in the two groups. Considering the results of Experiment 1 and the near significant main effect of status in Experiment 2, further analysis of the pattern across trials was carried out.

Next, a 3 (trials 1–3)×2 (status: satiated or withdrawn)×2 (condition: NRN or NNN) mixed-design ANOVA was used to investigate sorting rate patterns in detail across the trial series. This analysis of the sorting rates showed main effects of trial, status, and group ($F(2,80)=60.2$, $p<0.001$; $F(1,40)=9.9$, $p<0.01$; $F(1,40)=4.3$, $p<0.05$, respectively). As previously, there was an increase in sorting rate across trials and sorting rates were lower in the withdrawal condition. The overall sorting rate was higher in group NRN, presumably reflecting the effect of including the rewarded trial. The trial by group interaction was significant ($F(1.6, 63.9)=5.8$, $p<0.01$) but neither the trial by status, group by status, nor the trial by status by group interactions reached significance ($F(1.6,63.9)=2.51$, $p=0.1$; $F(1,40)<1$; $F(1.6, 63.9)=1.1$, $p=0.33$). Critically, however, there was a significant trial by status interaction when the quadratic trend across trials was considered ($F(1,40)=5.68$, $p<0.05$).

Figure 3a illustrates the trial by status interaction: the interaction on the quadratic trend repeated that which was

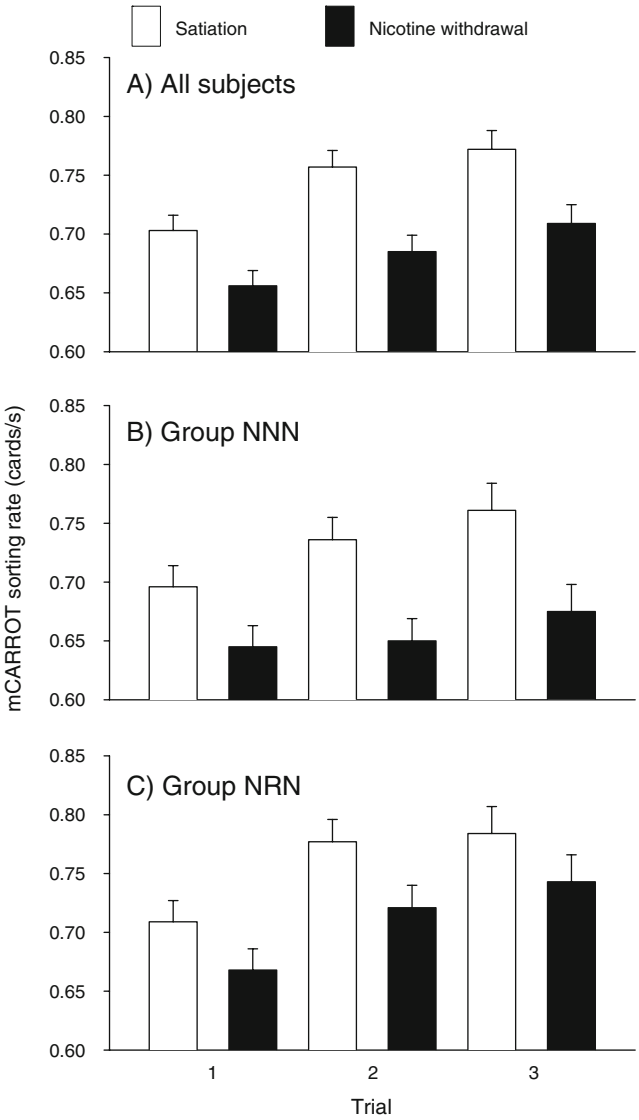


**Fig. 3** Mean (±SE) sorting rates across trials in Experiment 2 averaged over all subjects (**a**) and groups NNN (**b**) and NRN (**c**) under conditions of withdrawal and satiation. Participants in the withdrawal condition had abstained from smoking overnight; participants in the satiation condition were asked to smoke normally and smoke a cigarette immediately before attending for the experiment. Each trial lasted 5 min and group NNN completed all three trials nonrewarded. Sorting for group NRN was rewarded during the second trial. During rewarded sorting, ten pence (0.1 GBP) was earned for every five cards correctly sorted. Standard error bars based on SPSS Estimated Marginal Means subcommand

observed in Experiment 1 and appears again to be responsible for status effects on the derived measure. Further exploration used pairwise *t* tests in the first instance. Separate comparisons of trials 1 and 2, 1 and 3, and 2 and 3 for withdrawn and satiated subjects yielded significant differences in each case ($ts(21)>2.3$, $ps<0.05$). The difference between withdrawal and satiation was also significant at each trial ($ts(42)>2.5$, $ps<0.05$). Therefore, to clarify the source of the trial by status interaction on the quadratic trend, the interaction was examined with three 2×2 ANOVAs using trials 1 and 2, 1 and 2, and 2 and 3. There was a significant trial by status interaction when trials 1 and 2 were considered ($F(1,42)=4.8$, $p<0.05$) but not when trials 1 and 3 and trials 2 and 3 were analyzed ($F(1,42)=1.35$, $p=0.25$ and $F(1,42)<1$, respectively). Thus, although significant in each case, the increase in sorting rate between trials 1 and 2 in withdrawal was less than the increase observed in satiation. The absence of the three-way (trial by status by group) interaction in the overall analysis indicates that this effect was present in both groups NNN and NRN; the means for these groups are shown separately in Fig. 3b, c. However, the clearer difference between withdrawal and satiation for group NNN than group NRN suggests that differences in trends across trials may be more readily observed without the introduction of reward.

Third, the difference between groups NNN and NRN across trials indicated by the significant trial by group interaction that was found in the main analysis is shown in Fig. 4. Student's *t* tests at each trial showed a significant difference at trial 2 ($t(42)=2.6$, $p<0.05$) but not at trials 1 and 3 ($t(42)=0.94$, $p=0.35$; $t(42)=1.8$, $p=0.08$, respectively). Thus, the two groups were closely matched in trials 1 and 3 but group NRN sorted considerably faster than group NNN on their rewarded trial, regardless of their nicotine status.

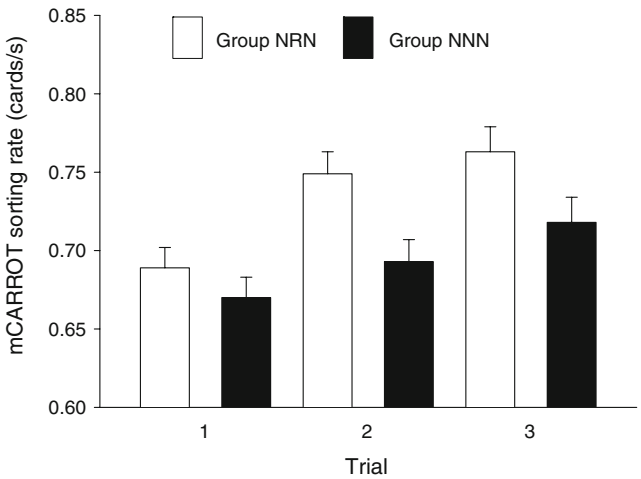


**Fig. 4** Mean (±SE) sorting rates across trials in Experiment 2 for subjects in groups NNN and NRN. Each trial lasted 5 min and the data point for each trial is averaged over equal numbers of participants in satiation and withdrawal. Group NNN completed all three trials nonrewarded. Sorting for Group NRN was rewarded during the second trial. During rewarded sorting, ten pence (0.1 GBP) was earned for every five cards correctly sorted. Each trial lasted 5 min. Standard error bars based on SPSS Estimated Marginal Means subcommand

Finally, a 2 (status: satiated or withdrawn)×2 (group: NNN or NRN) ANOVA was carried out on the data from trial 2 to determine whether or not the larger effect of reward seen under withdrawal in Experiment 1 would be replicated. This showed significant effects of status ($F(1,40)=13.8$, $p<0.01$) and group ($F(1,40)=8.44$, $p<0.01$) but no status by group interaction ($F(1,40)<1$). Thus, although the effect of reward was numerically larger in withdrawal than in satiation, this difference was not reliable.

## Discussion

Experiment 2 replicated key findings from Experiment 1. First, the mCARROT sorting rates increased across trials and second, the pattern of sorting rate across trials varied critically as a function of smoking status. Participants' sorting rates increased less quickly under withdrawal than under satiation. These effects led to a near significant effect ($p=0.068$) of smoking status when the difference between the sorting rate on trial 2 and the average sorting rate on trials 1 and 3 was considered. The absence of a group by status interaction on this measure indicated that this effect occurred independently of the introduction of a reward on trial 2. Analysis of the specific quadratic trend across trials showed significant differences between satiation and withdrawal, further supporting the conclusion that withdrawal produces a slower increase in sorting rate across trials. Third, overall sorting rates were once again slower under withdrawal than in satiation. Finally, although our procedure once again produced a reliable effect of reward, we did not find any indication that reward was more effective in satiation than in withdrawal. Additionally, we did not repeat the finding of Experiment 1 where withdrawal was associated with an increase in reward responsivity, an effect opposite to the one we expected. Therefore, this puzzling result from Experiment 1 must be treated with caution. Finally, in Experiment 1, the effect of withdrawal on overall sorting rate was subject to an interaction with testing order such that participants who were tested under withdrawal and then under satiation had a lower sorting rate under withdrawal than under satiation but those tested in the reverse order did not. This could have implied that the effect of withdrawal on sorting rate was confounded with an increase in sorting rate between experimental sessions. However, Experiment 2, which used a fully between-subjects design, provided a clearer demonstration of the effect of withdrawal on sorting rate.

## Overall discussion

The aim of the present research was to determine the extent to which the previously reported effect of nicotine withdrawal on reward responsivity (Al-Adawi and Powell 1997; Powell et al. 2002) could be attributed to reward responsivity per se. In two studies, we found that nicotine withdrawal was associated with a reduction in overall sorting rates in the mCARROT task and that the increase in sorting rate typically seen over a series of mCARROT trials was less marked under withdrawal than under satiation. Because withdrawal reduces the rate at which participants approach their asymptotic sorting rates, derived measures of reward responsivity such as those used by Powell et al. (Al-Adawi and Powell 1997; Powell et al. 2002) are actually measuring two processes. The derived measure adds together any effect of withdrawal on reward responsivity and any effect of withdrawal on the pattern of change across trials that occurs independently of the introduction of reward. When reward responsivity was assessed using a counterbalanced design to control the serial position of the rewarded trials and nonrewarded trials, withdrawal did not reduce reward responsivity (Experiment 1). Instead, there was a just significant effect in the opposite direction. However, we do not consider this result as reliable because there was no evidence for the same pattern in Experiment 2 and because the result from Experiment 1 was only significant when the order variables were included in the analysis. In fact, Experiment 2 provided no evidence for a difference in the effect of withdrawal on the pattern across trials whether or not a reward was included in the second trial of the series.

Perhaps, this failure to find an independent effect of withdrawal on reward responsivity, apart from that due to an effect on changes in sorting rate across trials, was due to the differences between the CARROT and mCARROT (trial duration, difficulty, and penalty for errors). Procedural differences between the CARROT and mCARROT were discussed earlier and one issue raised was that the reward effect may have been too robust to be reduced by withdrawal. This leads to the possibility of type II error secondary to small sample sizes. In previous studies, samples of 24–26 participants were tested in the CARROT under withdrawal, so our experiments should have had sufficient power to detect any effects that existed but this argument rests upon the assumption that our reward effects were as easy to disrupt as those previously observed. It may be that, in order to observe a direct effect of withdrawal on response contingent rewarded behavior, the reward effect has to be present but below some threshold of strength. An argument about lack of power cannot, however, be used against the positive findings from these studies. In the mCARROT, the dominant influence of nicotine withdrawal on performance was on the overall sorting rate and the change in sorting rate that occurred across a trial series rather than on the response to a financial incentive. In summary, our results showed that participants in withdrawal did not engage with the mCARROT task as effectively as satiated participants did, independently of whether performance contingent reward was used. This was shown in the overall sorting rates and on the rate at which participant's performance improved with practice. Both of these were reduced in withdrawal.

In conclusion, it is worthwhile considering how the observed effect of withdrawal on sorting rates and changes in sorting rate across trials should be interpreted. We began with a focus on the effects of nicotine withdrawal on reward-related processes but nicotine withdrawal has also been associated with a variety of cognitive effects, disturbances of mood, and motor deficits (e.g., Hughes 1992; Jarvis et al. 1998). One possibility is that nicotine withdrawal produces deficits in one or more cognitive, mood, or motoric functions and this impacts on behavior in tasks such as the CARROT and mCARROT. For example, withdrawal can produce deficits in attentional tasks and nicotine can improve performance in these (Koelega 1992). It, therefore, seems plausible to suggest that improvements over a series of trials in the mCARROT task (i.e., practice effects) would be most marked after recent nicotine intake, in contrast with performance during withdrawal, and this could be due to attentional mechanisms. It is also possible that some or all of the apparently diverse effects of nicotine withdrawal are based upon disruption in the activity of the reward mechanisms consequent to withdrawal (e.g., Koob and Le Moal 2005).

We began by highlighting a model of nicotine reward that emphasized nicotine's indirect reinforcing action, based upon modification of the effects of other reinforcers. Support for this idea has come from animal studies in which nicotine withdrawal has been shown to reduce the effectiveness of electrical brain stimulation reward in animals (Epping-Jordan et al. 1998) and from the research of Powell et al., based on an analogous human preparation. Although the current results suggest that reward responsivity in humans was not reduced by withdrawal, differences in the methods of the CARROT and mCARROT tasks could easily account for this failure to find an effect. Nevertheless, this study did reveal a novel effect of nicotine withdrawal on improvement in a motor task over a series of trials and an overall effect of withdrawal on performance.

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