Repeated intermittent oral amphetamine administration results in locomotor tolerance not sensitization

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Abstract

Background: The phenomenon of locomotor sensitization to injected amphetamine is well-characterised. The increased locomotor activity found acutely is enhanced with repeated intermittent treatment. This effect arises due to hypersensitization of the dopaminergic system and is linked to drug addiction. A clinical population exposed to chronic repeated intermittent amphetamine treatment such as is found for Attention Deficit Hyperactivity Disorder (ADHD) may be expected to be more at risk of addiction following this treatment. However, evidence suggests the opposite may be true. This suggests the route of administration may determine the direction of effects.

Aims and method: We aimed to establish how an oral amphetamine treatment regimen, similar to that used in ADHD impacts on locomotor activity, specifically whether tolerance or sensitization would arise. Healthy hooded lister rats were given amphetamine (2 mg/Kg, 5 mg/Kg and 10 mg/Kg) or a vehicle solution once daily for 4 weeks with a 5 day on, 2 day off schedule. Locomotor activity was measured on first day of treatment to establish the acute effects and on the final day of treatment to examine the chronic effects.

Results: As expected acute doses of amphetamine increased locomotor activity, although this only reached statistical significance for the 5 mg/Kg and 10 mg/kg doses. By contrast, after chronic treatment, animals administered these doses showed reduced activity indicating drug tolerance, rather than sensitization had occurred.

Conclusion: We suggest that the route of administration used in ADHD, which results in more stable and longer duration drug levels in the blood results in tolerance rather than sensitization and that this effect could explain the reduced likelihood of substance addiction in those treated with psychostimulants for ADHD.

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Keywords

Amphetamine, locomotor sensitization; tolerance; Attention Deficit Hyperactivity

Disorder

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Introduction

The phenomenon of locomotor sensitization, that is, the enhancement of drug-induced locomotor activity with repeated intermittent drug administration, was established almost 100 years ago [1]. The majority of studies have focused on amphetamine and cocaine and the effects of these psychostimulants are well-characterised [2]. At low-to-moderate doses, injected amphetamine results in an increase in locomotor activity when given acutely. When administration is repeated intermittently, this effect sensitizes with each successive dose [3]. There is strong evidence that this sensitization is caused by hypersensitivity within the dopaminergic systems [3]. Furthermore, the adaptation responsible for increases in the psychomotor response is also thought to underlie the sensitization of the incentive motivational properties of the drug which may underpin certain aspects of drug addiction [4, 5] and treatment regimens that cause locomotor sensitization are also associated with increased drug self-administration [6].

Given the established link between repeated amphetamine administration and sensitization to the both the locomotor and incentive value of the drug, it may be expected that those with any medical condition treated intermittently with repeated doses of amphetamine may be more at risk of developing drug addictions [7]. Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common behavioural disorders affecting around 6% of children and 3% of adults worldwide [8, 9]. It is most commonly treated using pharmacotherapy, and the use of the psychostimulant drugs amphetamines and methylphenidate is common [10]. However, contrary to what might be expected, research suggests that whilst

unmedicated children with ADHD have a significantly increased risk for any substance use disorders compared to those without ADHD, those who are medicated actually have a reduced risk versus those who are unmedicated [11]. One possible explanation for the lack of sensitization-related effects following repeated psychostimulant treatment in ADHD is the route of administration and duration of treatment. Studies in rodents that typically evoke locomotor sensitization usually employ repeated intraperitoneal (i.p) injections [6]. However, twice daily subcutaneous injections of amphetamine [12] have been found to produce tolerance, i.e. a reduced responsiveness to the drug in rodents. Similarly, use of a mini-pump to provide continuous infusion of amphetamine is also associated with tolerance to its effects [6, 13]. Work from other species also shows the importance of route of administration and suggest that routes which result in a more stable and constant level of amphetamine in the blood are associated with tolerance rather than sensitization [14]. Oral administration is known to result in a slower initial rise in blood plasma levels of the drug and more sustained concentration than injections [15]. The heightened concentrations also persist for a longer duration with oral administration [16]. It is consequently possible that the oral route of administration used in the treatment of ADHD results in a blood plasma profile which more readily resembles that producing tolerance rather than sensitization, and hence the latter rather than the former ensues. However, to date no research has examined the impact of a therapeutically-relevant oral treatment regimen on locomotor activity in the rat. Therefore, the present study aimed to establish whether oral administration of amphetamine, given chronically, as would be the case for ADHD, results in locomotor sensitization or tolerance. Based on the reasoning above, we hypothesized

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that chronic administration would result in tolerance to the psychomotor effects of amphetamine.

Subjects and methods

All experiments were approved by the Institutional Ethical Review Committee at the Open University, where the work took place (The Animal Welfare and Ethics Board) in advance. Work was also conducted with the authority of the appropriate U.K. Home Office Licenses and adhered to guidelines set out in the Animals [Scientific Procedures] Act (1986), EU Directive 86/609/EEC, and the "Guide for the care and use of Laboratory Animals" (NIH publication, 8th ed., The National Academies Press, Washington, 2011).

Male Hooded Lister rats were used, bred in-house as part of an on-going breeding colony, and aged six weeks at the start of experiments. In all cases, the individual rat was deemed the experimental unit. Female rats from within the colony were used for different research and, therefore, there was no animal wastage. Animals were housed with bedding and tubing in groups of 2 – 3, with standard lab chow (RM3 diet, Special Diet Services, Witham, UK) and water available ad libitum within the home cage. Cages were kept in scantainers held at a temperature of 21-23 °C, and humidity of approximately 50 %. The holding room was on a 12-h reverse dark-light cycle with lights turning on at 8 pm. After behavioural work was complete, animals were used for other experiments prior to sacrifice, therefore ensuring that as much data was obtained as possible from the cohort.

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Amphetamine (as d-amphetamine sulphate; Sigma Aldrich, UK) was prepared as a stock solution in distilled water and frozen at ⁻20 °C until use. Immediately prior to use it was defrosted and diluted 1:10 into apple juice (Just Juice, DME, Middlesex, UK) to give the final concentration for oral administration. Drugs were administered orally rather than by injection to more closely reflect how these drugs are taken by humans [15]. A vehicle control was also used, consisting of the same volume of distilled water, also previously frozen, diluted 1:10 into apple juice immediately prior to use. Dosing was achieved using a pipette [17], administering a volume of 1 µL/g (i.e. a rat of 100 g received 100 µL). This method of administration allows precise administration in the microlitre range, and has fewer health risks compared to oral gavage, which can result in damage to the oesophagus, or accidental drug delivery to lungs [18]. Prior to chronic treatment animals were habituated to oral administration using 200 µl of apple juice for 5 days. Drugs were then administered every day for 4 weeks (excluding weekends) resulting in animals receiving a total of 20 doses over 28 days as is used clinically for ADHD [19]. All treatment took place in the holding room, after daily weighing of the rats (to determine dose and monitor health status), at the start of the dark phase.

Three doses of amphetamine were used: 10 mg/kg (N=16), 5 mg/kg (N=18), and 2 mg/Kg (N=16) to compare with a vehicle (N=16). These doses were selected to ensure clinical relevance. Doses of amphetamine that are used clinically range from 5 to 60 mg [20, 21] and these are thought to result in blood plasma concentrations between 120 and 140 ng/ml in people receiving treatment for ADHD [22, 23]. When administered orally to rats, a dose 0.067 mg/ml gives a peak plasma concentration of 4 ng/ml [16] and, therefore, assuming a linear scaling, a dose of 2 mg/Kg would

amount to a blood plasma level of approximately 120 ng/ml. It was on this basis that our lower dose was chosen. We then selected two higher doses to allow comparison with other existing literature. Whilst this approach makes assumptions about linear scaling, it is generally accepted that the use of blood plasma levels is preferable to extrapolation on a milligram per kilogram basis from clinical doses when translating from humans to laboratory animals [15]. The drug treatment was performed blind, with randomly assigned letters representing each group, and dose was only revealed after completion of all analyses.

Locomotor activity was measured using $40 \times 40 \times 35$ cm plexiglass activity monitoring chambers (Med-Associates, Sandown Scientific, Hampton, Middlesex, UK) that automatically measure activity using horizontal beam breaks. Animals were habituated to the chambers for 15 min on two consecutive days immediately prior to the first locomotor activity test to ensure familiarity with the chambers. Locomotor activity was then recorded for 1-h on the first and the final day of chronic treatment beginning 30 min after administration. This period was chosen to ensure that the peak psychostimulant activity, which occurs approximately 1-h post drug administration, was recorded (Kuczenski & Segal, 2002; Martínez-Clemente et al, 2013; Sakai et al., 1983). Activity chambers were cleaned after each test, before a new animal entered the chamber, to remove all olfactory cues. Data were stored in 5-min epochs for offline analysis.

Statistical analysis

The number of horizontal beam breaks was checked for normality using the Kolmogorov-Smirnov test and measures of skewness and kurtosis and were

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subsequently deemed suitable for use with parametric tests. Analysis was then conducted to investigate both the acute and chronic effects of oral amphetamine on locomotor activity. Acute effects were examined by analysing the locomotor activity from the first day of treatment using a Mixed Measures ANOVA with DOSE as the between-subjects factor and TIME as the within-subjects factor. Chronic effects of amphetamine were examined by repeating the same analysis on the data from the final day of treatment. In both cases, where a significant interaction effect was found a series of restricted ANOVAs were conducted to examine what drove this interaction. Restricted ANOVAs were carried out for all possible group combinations (vehicle vs 2 mg/Kg, vehicle vs 5 mg/Kg, vehicle vs 10 mg/Kg, 2 mg/Kg vs 5 mg/kg, 2 mg/Kg vs 10 mg/Kg, 5 mg/Kg vs 10 mg/Kg), with any significant interaction in these restricted ANOVAs reported as potentially underlying the interaction in the main analyses. Additionally, a second analysis was used to establish whether there was any change in sensitivity to each dose between the first and final day. For this we conducted a Mixed-Measures ANOVA with DAY and TIME as within-subjects factors.

In reporting the outcome of statistical tests, we have provided both the effect size and observed power in addition to statistical significance. The effect size provides a measure of the magnitude of the difference between groups in an analysis and it can be considered the main finding of a quantitative study [24]. The p-value provides information about whether an effect exists but the effect size reveals the size of the effect and it is becoming increasing recognised that both values should be reported [24, 25]. For ANOVA analyses, partial-eta squared (η^2) is provided for effect size where values of 0.01, 0.06, and 0.14 indicate small, medium, or large effects and the overlaps outlined above. Whilst all measures of effect size reflect the proportion of

variance in the dependent variable associated with different levels of the independent variable, this measure of effect size, in comparison to eta-squared (η^2), uses a denominator which contains only the variance attributable to the effect of interest plus the error [26] and has been suggested to be most useful when researchers may wish to compare across studies with slightly different designs [27]. Observed statistical power is provided to show the probability of rejecting a false null hypothesis. It is generally accepted that power should be at least 0.8. Finally, for all repeated measures ANOVAs, if the assumption of sphericity was violated, the Greenhouse-Geisser [28] results were reported.

Results

Effects of acute oral amphetamine

The acute effects of amphetamine treatment are shown in Figure 1 alongside the chronic effects. Analyses of the acute effects demonstrated that there was a significant main effect of TIME ($F(4.33, 268.39)=8.61$; $p<0.001$; $\eta^2=0.122$, Power=0.999) with an overall decline in activity with time. Within-subjects difference contrasts revealed significant decreases between all consecutive time points except between 20 and 35 minutes when the main effect of time is considered (i.e. collapsed across doses). However, it is notable that the higher doses show less decline. There was also a main effect of DOSE ($F(3, 62)=11.28$; $p<0.001$, $\eta^2=0.353$, Power=0.990). Amphetamine produced a dose-dependent elevation in activity relative to control. For dose, post-hoc Tukey tests revealed that the 5 mg/Kg and 10 mg/Kg group differed significantly from the control group (5 mg/Kg p=0.001; 10 mg/Kg p<0.001) and the 2 mg/Kg group (5 mg/Kg p=0.039; 10 mg/Kg p=0.001). The control and 2mg/Kg group did not

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differ from each other. In addition, there was a significant TIME x DOSE interaction $(F_{(12.97, 268.39)}=3.03$; $p<0.001$; $\eta^2=0.128$, Power=0.994). Restricted Mixed-Measures ANOVAs as described above revealed that the significant interaction between time and dose was present for all group comparisons except the vehicle and 10 mg/Kg groups and the 5 mg/Kg and 10 mg/Kg group.

Effects of chronic oral amphetamine

The chronic effects of amphetamine treatment are also shown in Figure 1. On the final treatment day, analyses demonstrated that there was a significant main effect of TIME $(F_{(6.00, 371.13)}=62.92$; $p<0.001$; $\eta^2=0.504$, Power=1.00) but no main effect of DOSE $(F_{(3, 62)}=2.33$; $p=0.083$, $\eta^2=0.101$, Power=0.560). As expected, across time there was an overall decline in activity with within-subjects difference contrasts revealing significant differences between all consecutive time points. In addition, there was a significant TIME x DOSE interaction $(F_{(18.00, 371.13)}=2.21$; $p=0.003$; $\eta^2=0.097$, Power=0.989). Restricted Mixed-Measures ANOVAs revealed that significant interactions were present for any comparison that includedthe 2 mg/Kg group. Figure 2 suggests that this is because this group has a steeper initial decline in activity.

Figure 1 shows the different treatment conditions across the first and final day. For the vehicle condition, there was no significant main effect of DAY $(F_{(1, 165)}=3.98$; $p=0.065$; $\eta^2=0.210$, Power=0.463) but there was still a main effect of TIME $(F_{(11, 165)}=24.13$; $p<0.001$; $\eta^2=0.617$, Power=1.000) as described for both days above i.e. an overall decline with time in the chamber. There was no significant DAY x TIME

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interaction (F(1, 165)=1.41 p=0.173; η^2=0.086, Power=0.728). For the 2 mg/Kg condition, there was no significant main effect of DAY (F(1, 165)=0.37; p=0.551; η^2=0.024, Power=0.088) but there was still a main effect of TIME (F(3.27, 90.54)=29.92; p<0.001; η^2=0.666, Power=1.000) as described above. There was a significant DAY x TIME interaction (F(1, 165)=3.33; p<0.001; η^2=0.182, Power=0.992). The lack of a main effect of DAY for both the control and 2 mg/Kg group indicates that there was no difference between responsiveness to the vehicle or low dose of amphetamine following chronic treatment. By contrast, for the 5 mg/Kg condition, there was a significant main effect of DAY (F(1, 187)=10.32; p=0.005; η^2=0.378, Power=0.857) with activity reduced on the final day compared to the first, indicating a drug tolerance. There was also still a main effect of TIME (F(4.36, 81.60)=9.55; p<0.001; η^2=0.360, Power=1.000) with an overall decline with time in the chamber. There was a significant DAY x TIME interaction (F(1, 165)=6.34; p<0.001; η^2=0.272, Power=1.00). Finally, for the 10 mg/Kg condition, there was also a significant main effect of DAY (F(1, 165)=9.70; p=0.007; η^2=0.392, Power=0.829) with activity reduced on the final day compared to the first, again indicating drug tolerance with chronic treatment. There was also still a main effect of TIME (F(3.06, 47.56)=3.54; p=0.021; η^2=0.191, Power=0.755) as described above. There was a significant DAY x TIME interaction (F(3.17, 47.56)=3.30; p=0.026; η^2=0.180, Power=0.732).
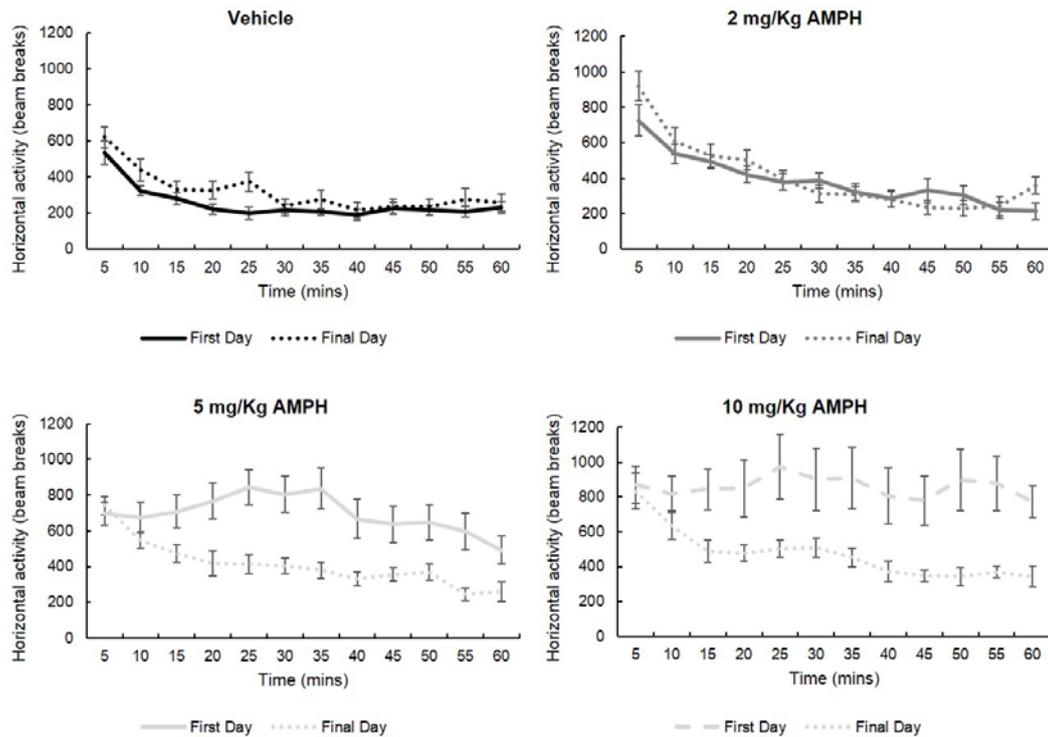
Figure 1: A comparison of effects on the first and final day for the different treatment groups. On the first day of amphetamine treatment, there is a significant increase in activity compared to the control group in those treated with 5 and 10 mg/Kg amphetamine. On the final day of amphetamine treatment there was no longer any significant differences between the four groups.

Discussion

We reasoned that the locomotor sensitization effects frequently observed in rodents following psychostimulant administration were dependent on the route administration and that routes which typically result in a more constant blood plasma level of the drug are likely to result in tolerance rather than sensitization. Specifically, we hypothesized that treatment with amphetamine using a regimen similar to that found in ADHD treatment i.e. chronic oral treatment, would result in tolerance rather than sensitization. Our results largely support this hypothesis. We found that when administered acutely, the higher doses of amphetamine resulted in an increase in

locomotor activity, which is also seen with acute administration of injected amphetamine. The lower, but still therapeutically relevant dose (2 mg/Kg) did not significantly increasing locomotor activity. Critical to our hypothesis, however, we found that after 20 days of oral administration 5 days a week, both higher doses (5 mg/Kg and 10 mg/Kg) induced a significant tolerance in responsiveness, whilst there was no change in sensitivity to the lower dose or vehicle.

These results support the premise that dosing regimens which result in more constant blood plasma levels of psychostimulants are more likely to induce tolerance than locomotor sensitization. Oral administration is known to result in a slower initial rise in blood plasma levels of the drug and more sustained concentration than injections [15]. The heightened concentrations also persist for a longer duration with oral administration [16]. These results are in line with the previous work indicating that those treated with amphetamine for ADHD are at less risk of substance use disorders [11] because the psychomotor effects are known to mirror the effects of incentive motivation and, therefore, a reduction in psychomotor responsiveness, may also result in a reduction in incentive motivation [4-6]. It should be noted that some previous work in humans has also failed to find evidence for locomotor sensitization following oral amphetamine treatment in healthy individuals [29] and that the results of the current study are in line with this. However, other studies have indicated such sensitization may occur [30, 31]. In all cases, and in marked contrast to the present study, treatment was only given for 4 days and, therefore, not for the duration expected for ADHD treatment. It may therefore be that the route of administration and the overall period of administration dictate the effects seen for amphetamine.

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Although our results are in line with some research with clinical and healthy human populations investigating amphetamine, there is a small amount of evidence from animal work showing cocaine, also a psychostimulant, can cause sensitization even when administered orally. When administered via gavage at relatively high doses for 16 consecutive days, cocaine has been found to induce sensitization [32]. However, others have not been able to demonstrate robust sensitization to oral cocaine [33]. Given the mixed results for cocaine, and the different synaptic mechanisms of the two drugs, it is important to recognise that the results presented here may not generalise to psychostimulants other than amphetamine and that other drugs should be examined in future.

Despite the findings showing that chronic treatment with amphetamine can result in tolerance rather than sensitization, some caveats of the current study must be acknowledged. Firstly, whilst every effort has been made to ensure doses are therapeutically relevant and administered using an appropriate method to best mirror human use, we did not measure blood plasma levels, something that should be considered in future research. Secondly, we did not directly compare to a group of rats treated with injections of amphetamine, choosing instead to compare to the existing literature for this. It is possible that, in our hands, that regimen would not have elicited locomotor sensitization. However, given the similarity of the acute effects seen here with previous studies, this seems unlikely. Finally, the age of the animals in the present study would limit the applicability of the results to children with ADHD. However, given that the condition is now accepted to be present in adults as well children and the two cohorts are deemed distinct [34] these results do still have relevance to the clinical population.

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Conclusions

This study has shown that acute administration of oral amphetamine can result in the increased locomotor activity commonly seen when amphetamine in administered by i.p injection. Importantly, we have also shown that after chronic oral treatment, the raised locomotor activity seen acutely is lost and instead a tolerance to the drug effects are found. This is in marked contrast to the effects of chronic i.p. treatment and supports previous work that indicates the mode of administration and the duration of drug effects is important. Critically, the present study attempted to emulate the treatment regimen used in ADHD and, therefore, the results can feed into a growing body of literature attempting to understand the relationship between this common condition, so often treated with psychostimulants, and substance addictions. Based on the present results, we suggest that chronic oral amphetamine treatment may actually protect against future substance addiction via the induction of tolerance.

Declaration of Conflicting Interests

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