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# Varying the rate of intravenous cocaine infusion influences the temporal dynamics of both drug and dopamine concentrations in the striatum

*Ellie-Anna Minogianis, Waqqas M. Shams, Omar S. Mabrouk, Jenny-Marie T. Wong, Wayne G. Brake, Robert T. Kennedy, Patrick du Souich and Anne-Noël Samaha*

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Review timeline:

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| Submission date: | 08 December 2018 |
| Editorial Decision: | 13 February 2018 |
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| Editorial Decision: | 25 March 2018 |
| Revision Received: | 26 March 2018 |
| Accepted: | 27 March 2018 |

Editor: Michel Barrot

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1st Editorial Decision 13 February 2018

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Dear Dr. Samaha,

Your manuscript has now been reviewed by two external reviewers and one of our senior section editors. While they indicate that your experiments and data have some interest, they also raise major limitations that will require a major revision of the manuscript if we are to proceed with the paper here at EJN. In particular, the question of the cocaine dose (rather high, in fact over what is classically used in the field) as well as the time-course (short, thus preventing an assessment of the delay before returning to or below baseline) should be discussed; the choice of dorsal striatum for recordings (which may be related to technical limitations) should be more criticially discussed; adding areas under the curve for cocaine and DA (as added panels in the corresponding figure) should be considered; it sounds inappropriate to detect cocaine in its absence, please clarify/correct (cf Fig2, provide also the appropriate time-course statistics in results). The other comments from both reviewers should also be constructively addressed. At editorial level we would also suggest: 1) to fusion fig 3 and 4 in a single figure (drawings on the left, graphs on the right) to gain space, idem for Fig5 and 6; 2) indicate in methods how animals were killed; 3) To comply with EJN guidelines, please replace all bar graphs with more informative scatter plots representation or similar, giving access to individual data (see http://onlinelibrary.wiley.com/doi/10.1111/ejn.13400/full and EJN publications of past year for examples).

Thank you for submitting your work to EJN.

Kind regards,

John Foxe & Paul Bolam
co-Editors in Chief, EJN

Reviews:

Reviewer: 1 Vedran Lovic (University of Calgary, Canada)

Comments to the Author
EJN-2017-12-25168(ADX)

Varying the rate of intravenous cocaine infusion influences the temporal dynamics of both drug and dopamine concentrations in the striatum

There are numerous factors that influence the reinforcing effects and addiction potential of addictive drugs. All addictive drugs potentiate dopamine (DA) neurotransmission albeit through different mechanisms. A number of addictive drugs can have multiple routes of administration. For example, cocaine can be ingested (which is very rare), insufflated, injected, or smoked. These routes of administration, irrespective of the dose (and even if doses are adjusted), produce different effects. Furthermore, even within one mode of administration (e.g., intravenous – i.v.), drugs can be administered with varying speed. It has been hypothesized, and limited evidence has been gathered, that faster routes of administration differentially recruit relevant circuitry (e.g., the striatum) and increase the addiction potential of a particular drug, whether it's a stimulant like cocaine, or an opioid like fentanyl. However, there are remaining questions of whether faster routes of drug administration produce greater peak concentrations of a particular drug itself or DA. Furthermore, there is uncertainty whether these peak

concentrations are reached in a shorter period of time following fast vs. slow drug administration.

Here authors were interested in examining brain cocaine and DA dynamics following fast (5s), medium (45s), or slow (90s) i.v. infusion of cocaine. There is a solid rationale for conducting the study presented in this manuscript. Answering the posed questions is technically challenging as minute temporal resolution is necessary. The authors found speed of i.v. cocaine delivery did not change peak concentrations of cocaine or DA (even though, the trend was there) . However, peak concentrations were reached faster following 5s i.v. infusion. In addition, other chemical species were also measured and were found not to be changed by the speed of delivery. With respect to locomotor activity, speed of infusion did not alter total distance traveled but cocaine-evoked locomotor activity was initiated with shorter latency when cocaine was delivered in 5s. These findings are novel and highly relevant for our understanding of drug pharmacodynamics and addiction liability.

Introduction.
The introduction is exceptionally well written. It is succinct and contains relevant background information. The rationale for the study is elegantly presented. In fact, the entire manuscript is well written – it is once of the clearest and well put manuscript I have read in a long time.

Methods.
I am somewhat surprised by the dose of cocaine used here. Typical i.v. self-administration doses range from 0.05-0.75 mg/kg. 2.0 mg/kg is a rather large dose. Perhaps lower doses presented detection challenge. It would be useful to briefly rationalize this rather high dose.

Figure 1. depicting the experimental outline is fine but it could be aesthetically improved. This is just a suggestion.

I am somewhat surprised that sampling was done for only 15 minutes post cocaine infusion. It would have been useful to see how long it took for both cocaine and DA to return to baseline levels following different speeds of infusion.

Results

Would it be useful to have calculate and statistically assess of area under the curve for cocaine and DA for different infusion speeds?

Discussion

The discussion is well written summarizing the rationale for the study and main findings, placing them in the context of existing literature. Overall, I enjoyed reading it.

Reviewer: 2 Pascal Romieu (CNRS, France)

Comments to the Author
Dear colleagues,

In the manuscript presented by Minogianis et al., the authors were interested in the clarification of parameters leading to increased psychomotor (and potentially motivational) effects of high rates of cocaine intravenous infusion in comparison to low infusion rates. They analyzed, through rapid microdialysis then HPLC-MS/MS, cocaine and dopamine intra-striatal concentrations in rats after delivery of the same dose of cocaine in 5 versus 45 or 90 seconds and found that the main parameter involved in cocaine-induced dopamine increase and acute hyperlocomotion was the rate of increase of brain's cocaine concentrations, and consequently dopamine kinetics, rather than the level of the peak concentration of these compounds.
So the relevance of the study for the aims and scopes of EJN is certain. In addition, authors' guidelines were respected.
Albeit that the choice to repeat intravenous injections of cocaine in same rats rather than to use different rats for different rates of infusion could be open to discussion, the technical quality of the work is correct and experiments were rigorously performed, from a scientific standpoint.

In case of acceptance, however, some minor modifications and/or clarifications should be provided to the manuscript:
- Is there any evidence that a previous cocaine infusion does not alter effects of a subsequent one? If experimental data reveal a lack of effect, the authors should mention it; even show it in a supplemental figure or table.
- Limits of detection for cocaine and dopamine (p.12) should precise if they concern concentrations before or after derivatization.
- The authors should explain why, although cocaine was absent in the first solution (aCSF) where dialysis probes were immersed for determination of delay, they can detect cocaine (near 25 nM).
- Microdialysis probe locations, in fig. 3, should not indicate the tip segment devoid of membrane diffusion (in NAc) since no exchange is possible.
- The cocaine dose used in this study (2 mg/kg/infusion) is, contrary to what is written in discussion (p. 19), far from being 'within the range used in drug SA studies'. Actually, most of studies use a 0,175-0,75 mg/kg/infusion range (albeit dose is increased, in some cases, to 1,5).

However, a weakness affecting this manuscript is that the sole progress it provides to the global knowledge is the possibility to measure both cocaine and other compounds (such as dopamine) simultaneously in freely moving animals. But one could argue that different results other than those presented here would have been very strange, thus limiting the interest for the study. Moreover, many studies have already been published about effects of cocaine infusions rates. One of them, Ferrario et al (2008), also signed by two authors of the present manuscript (Samaha AN, Kennedy RT), described very similar results except that c-fos mRNA was measured but not locomotor activity (and cocaine

dosage). Finally, the link between these results and addiction is far from being obvious because nucleus accumbens should have been chosen for microdialysis instead of dorsal striatum. Although the latter is certainly involved in some neural processes related to addiction (formation and maintenance of habits), these processes develop gradually and need repetition of 'cued' instrumental conditioning. Neither acute psychomotor effects nor striatal dopamine increase are thought to reflect emotional/subjective cocaine effects such as euphoria (considering that these subjective effects are of prime importance to lead to addiction). So, comparing chronic repeated versus acute administrations of cocaine (for instance with implantation of bilateral cannulae to be used in distinct times) should have been performed in this study in order to perhaps provide new information.

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Authors' Response 15 March 2018

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Manuscript Number: EJN-2017-12-25168(ADX)

Title: "Varying the rate of intravenous cocaine infusion influences the temporal dynamics of both drug and dopamine concentrations in the striatum"
European Journal of Neuroscience

Editor Comments to Author:
Your manuscript has now been reviewed by two external reviewers and one of our senior section editors. While they indicate that your experiments and data have some interest, they also raise major limitations that will require a major revision of the manuscript if we are to proceed with the paper here at EJN. In particular, the question of the cocaine dose (rather high, in fact over what is classically used in the field) as well as the time-course (short, thus preventing an assessment of the delay before returning to or below baseline) should be discussed; the choice of dorsal striatum for recordings (which may be related to technical limitations) should be more critically discussed; adding areas under the curve for cocaine and DA (as added panels in the corresponding figure) should be considered; it sounds inappropriate to detect cocaine in its absence, please clarify/correct (cf Fig2, provide also the appropriate time-course statistics in results). The other comments from both reviewers should also be constructively addressed. At editorial level we would also suggest: 1) to fusion fig 3 and 4 in a single figure (drawings on the left, graphs on the right) to gain space, idem for Fig5 and 6; 2) indicate in methods how animals were killed; 3) To comply with EJN guidelines, please replace all bar graphs with more informative scatter plots representation or similar, giving access to individual data (see http://onlinelibrary.wiley.com/doi/10.1111/ejn.13400/full and EJN publications of past year for examples).

If you are able to respond fully to the points raised, we would be pleased to receive a revision of your paper within 12 weeks.

Thank you for submitting your work to EJN.

Kind regards,

John Foxe & Paul Bolam
co-Editors in Chief, EJN

Editorial Comments to Author:
Comments to the Author:
At editorial level we would also suggest:
1) cf Fig2, provide also the appropriate time-course statistics in results.

Reply:
Time-course statistics for Figure 2 have been included in the ms.
Changes to ms:
Materials and methods, under "Statistical analysis"

Changes in cocaine concentration as a function of time during the in vitro assay were analyzed using one-way analysis of variance (ANOVA).
Results, under Determination of microdialysis probe delay time in vitro
Once the microdialysis probes were placed into the cocaine solution, cocaine concentrations increased significantly from baseline levels (One-way ANOVA on minutes -10 to 26; $F(35,36) = 26.08$; $p < 0.05$). When the microdialysis probes were placed back into the solution that did not contain cocaine, drug concentrations significantly decreased (One-way ANOVA on minutes 0 to 42; $F(42,43) = 17.51$; $p < 0.05$).

2) to fusion fig 3 and 4 in a single figure (drawings on the left, graphs on the right) to gain space, idem for Fig5 and 6;

Reply:

Figures 3 and 4 have now been combined and renamed figure 3. Figures 5 and 6 have also been combined and renamed figure 4.

3) indicate in methods how animals were killed;

Changes to ms:

Materials and Methods, under "Histology"

Following sampling, animals were anaesthetized with isoflurane and decapitated. Brains were then extracted, frozen and stored at -80°C.

Materials and Methods, under "Psychomotor Activity Experiment"

At the end of the study, catheter patency was verified once again with Propofol and animals were immediately euthanized by decapitation while still under anaesthesia.

4) To comply with EJN guidelines, please replace all bar graphs with more informative scatter plots representation or similar, giving access to individual data.

Reply:

Individual data is now included in all bar graphs.

Reviewers' Comments to Author:

Reviewer #1:

Comments to the Author:

There are numerous factors that influence the reinforcing effects and addiction potential of addictive drugs. All addictive drugs potentiate dopamine (DA) neurotransmission albeit through different mechanisms. A number of addictive drugs can have multiple routes of administration. For example, cocaine can be ingested (which is very rare), insufflated, injected, or smoked. These routes of administration, irrespective of the dose (and even if doses are adjusted), produce different effects. Furthermore, even within one mode of administration (e.g., intravenous – i.v.), drugs can be administered with varying speed. It has been hypothesized, and limited evidence has been gathered, that faster routes of administration differentially recruit relevant circuitry (e.g., the striatum) and increase the addiction potential of a particular drug, whether it's a stimulant like cocaine, or an opioid like fentanyl. However, there are remaining questions of whether faster routes of drug administration produce greater peak concentrations of a particular drug itself or DA. Furthermore, there is uncertainty whether these peak concentrations are reached in a shorter period of time following fast vs. slow drug administration.

Here authors were interested in examining brain cocaine and DA dynamics following fast (5s), medium (45s), or slow (90s) i.v. infusion of cocaine. There is a solid rationale for conducting the study presented in this manuscript. Answering the posed questions is technically challenging as minute temporal resolution is necessary. The authors found speed of i.v. cocaine delivery did not change peak concentrations of cocaine or DA (even though, the trend was there). However, peak concentrations were reached faster following 5s i.v. infusion. In addition, other chemical species were also measured and were found not to be changed by the speed of delivery. With respect to locomotor activity, speed of infusion did not alter total distance traveled but cocaine-evoked locomotor activity was initiated with shorter latency when cocaine was delivered in 5s. These findings are novel and highly relevant for our understanding of drug pharmacodynamics and addiction liability.

Reply:

We thank Reviewer #1 for his/her positive evaluation of this manuscript and for highlighting the importance of the work.

1) Introduction: The introduction is exceptionally well written. It is succinct and contains relevant background information. The rationale for the study is elegantly presented. In fact, the entire manuscript is well written – it is one of the clearest and well put manuscript I have read in a long time.

Reply:

This is great to read. Thank you.

2) Methods: I am somewhat surprised by the dose of cocaine used here. Typical i.v. self-administration doses range from 0.05-0.75 mg/kg. 2.0 mg/kg is a rather large dose. Perhaps lower doses presented detection challenge. It would be useful to briefly rationalize this rather high dose.

Reply:

While many self-administration studies use lower doses of cocaine, we chose 2 mg/kg/infusion for two reasons. First, this allowed us to compare our findings to others where cocaine and/or dopamine levels were measured in the caudate-putamen following an acute intravenous cocaine infusion (Hurd et al., 1988; 1989, Ferrario et al., 2008). These studies used 1.5 – 2.0 mg/kg/infusion. Second, compared to a lower dose (i.e., 0.5 mg/kg), an acute intravenous infusion of 2 mg/kg cocaine evokes higher levels of immediate early gene expression (c-fos and arc mRNA) in the caudate-putamen (Samaha et al., 2004). Cocaine-induced increases in striatal dopamine levels contribute to immediate early gene expression. Thus, studying a 2 mg/kg dose ensured that we would detect a robust dopamine response with in vivo microdialysis.

Changes to ms:
Materials and Methods, under "In vivo Microdialysis Experiment"

A 2 mg/kg dose of cocaine is similar to doses used in prior studies that have measured cocaine or dopamine concentrations in the striatum using in vivo microdialysis (Hurd et al., 1998; Hurd & Ungerstedt, 1989; Ferrario et al., 2008). This dose also evokes robust immediate early gene expression in the dorsal striatum (Samaha et al., 2004).

3) Figure 1. depicting the experimental outline is fine but it could be aesthetically improved. This is just a suggestion.

Reply:
Figure 1 has been modified.

4) I am somewhat surprised that sampling was done for only 15 minutes post cocaine infusion. It would have been useful to see how long it took for both cocaine and DA to return to baseline levels following different speeds of infusion.

Reply:
It would certainly be useful to continue sampling past 15 minutes post-infusion, such that additional pharmacokinetic parameters could be obtained. However, sample analysis is costly. We limited the post-infusion sampling period to keep the financial costs of the study manageable. While not ideal, these are the kinds of practical considerations one must make when conducting such experiments. This being said, the central objective was to determine how variation in the speed of i.v. cocaine infusion influences cocaine and dopamine Cmax and Tmax values. The sampling period we used allowed us to meet this objective.

5) Results: Would it be useful to calculate and statistically assess the area under the curve for cocaine and DA for different infusion speeds?

Reply:
Certainly. However, the present findings cannot be used to accurately determine area under the curve values. As stated in our reply to comment #4, we limited the number of post-cocaine samples to 15 (1 sample/minute) for practical reasons. Cocaine and dopamine peaks were reached earlier with faster infusions. This means that there were more cocaine and dopamine samples taken after peak concentrations were reached following the 5-s infusion versus the 90-s infusion. For this reason, it would not be appropriate to compare area under the curve between the three different infusion speeds in this study. However, in Ferrario et al., (2008) we show that area under the curve, at least for dopamine, would be similar when cocaine is injected i.v. over 5 versus 100 s. We now mention this in the Discussion section of the manuscript.

Changes to ms:
Discussion, 3rd Paragraph

Our findings also agree with those of Ferrario et al. (2008) showing that infusing cocaine i.v. between 5 and 90 s does not produce large effects on peak dopamine concentrations in the striatum (or on area under the curve values for dopamine), but it produces significant differences in dopamine Tmax.

6) Discussion: The discussion is well written summarizing the rationale for the study and main findings, placing them in the context of existing literature. Overall, I enjoyed reading it.

Reply:
We are pleased that Reviewer #1 enjoyed reading our manuscript.

Reviewer #2:
Comments to the Author:
In the manuscript presented by Minogianis et al., the authors were interested in the clarification of parameters leading to increased psychomotor (and potentially motivational) effects of high rates of cocaine intravenous infusion in comparison to low infusion rates. They analyzed, through rapid microdialysis then HPLC-MS/MS, cocaine and dopamine intra-striatal concentrations in rats after delivery of the same dose of cocaine in 5 versus 45 or 90 seconds and found that the main parameter involved in cocaine-induced dopamine increase and acute hyperlocomotion was the rate of increase of brain's cocaine concentrations, and consequently dopamine kinetics, rather than the level of the peak concentration of these compounds. So the relevance of the study for the aims and scopes of EJN is certain. In addition, authors' guidelines were respected.

Reply:
We are pleased that Reviewer #2 highlights both the relevance of this work and its appropriateness for publication in the European Journal of Neuroscience.

1) Albeit that the choice to repeat intravenous injections of cocaine in same rats rather than to use different rats for different rates of

infusion could be open to discussion, the technical quality of the work is correct and experiments were rigorously performed, from a scientific standpoint. Is there any evidence that a previous cocaine infusion does not alter effects of a subsequent one? If experimental data reveal a lack of effect, the authors should mention it; even show it in a supplemental figure or table.

Reply:

As the Reviewer states, the three infusion rates were tested in a within-subjects design. This was done to minimize the number of animals used, as this is an ethical requirement. The question regards the possibility of carry-over effects. As is now reported in the revised manuscript, cocaine and dopamine concentrations returned to pre-cocaine baseline levels before each infusion. We took 5 baseline samples in the 5 minutes immediately prior to each cocaine infusion. Cocaine (Two-way ANOVA, main effect of Infusion Order; $F(2,18) = 1.42$, $p = 0.27$) and dopamine concentrations ($F(2,18) = 0.15$, $p = 0.86$) measured in the 5-min period prior to each of the 3 infusions were similar. This is also in agreement with a previous report, where two i.v. cocaine infusions (1.5 mg/kg/infusion) were given 90-min apart (Hurd et al., 1988). Hurd et al. (1988) report that cocaine concentrations declined towards the limit of detection prior to the second infusion. Their results also indicate that brain cocaine pharmacokinetics following the first and second bolus were similar, i.e. cocaine levels peaked at 10 minutes post-infusion, reached similar peak drug levels and were minimal within 60 minutes.

The observation that, under our conditions, cocaine and dopamine concentrations returned to baseline levels before each infusion is in accordance with cocaine's T1/2 in rat brain. Cocaine infusions were spaced 90 minutes apart. This is three to four times longer than cocaine's T1/2 in rat brain [approximately 20-30 minutes (Nayak et al. 1976; Hurd et al., 1988), including in the caudate-putamen specifically (Hurd et al., 1988)].

Changes to ms:

Methods, under "In vivo Microdialysis Experiment"

Cocaine infusions were spaced 90 minutes apart because this is 3-4 times longer than cocaine's T1/2 in rat brain (Nayak et al., 1976; Hurd et al., 1988). Thus, the 90-minute inter-infusion interval reduces the possibility of carry-over effects between infusions [see also (Hurd et al., 1988)].

Results, under "Varying the rate of i.v. cocaine delivery between 5 and 90 seconds significantly influences striatal cocaine and dopamine Tmax, but not Cmax"

All animals received all three intravenous cocaine infusions administered over 5, 45 and 90 s, in counter-balanced order. Baseline levels of cocaine and dopamine during the 5 minutes prior to each infusion were comparable (Cocaine: $F(2, 18) = 1.42$, $p = 0.27$; Dopamine: $F(2,18) = 0.15$, $p = 0.86$; data not shown). Thus, cocaine and dopamine concentrations returned to pre-cocaine baseline levels before each infusion, and there were no significant carry-over effects from one infusion to the next.

2) Limits of detection for cocaine and dopamine (p.12) should precise if they concern concentrations before or after derivatization.

Changes to ms:

Materials and Methods, under "High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)"

The limits of detection for cocaine and dopamine after derivatization were 0.291 and 0.086 nM, respectively.

3) The authors should explain why, although cocaine was absent in the first solution (aCSF) where dialysis probes were immersed for determination of delay, they can detect cocaine (near 25 nM).

Reply:

This is a background signal, due to a contaminant with a mass transition similar to cocaine at this specific retention time. The source of this background signal was not investigated since it did not interfere with our ability to reliably measure exogenous cocaine spikes. One could argue that the low resolution of the triple quadrupole mass spectrometer used (0.7 Da) may be a contributing factor.

We overcame this issue by taking the background contaminant signal into account when assigning our lower limit of quantification (LLOQ), which we estimated as 3X the signal generated in aCSF alone, or 23 nM. The baseline values in Figure 2 were all below 23 nM and thus we consider this to be "noise" rather than actual cocaine signal. It is only when the dialysis probe was placed in the cocaine-containing solution that cocaine levels rise above the LLOQ. We have now highlighted this in the manuscript.

Changes to ms:

Results, under "Determination of microdialysis probe delay time in vitro"

Of note, apparent cocaine concentrations do not start at or return to '0' when the probes are placed in the aCSF/ascorbic acid solution, before and after probe immersion in the cocaine solution. This background signal is likely produced by a contaminant with a similar mass transition to cocaine. We overcame this issue by taking the background contaminant signal into account when assigning the lower limit of quantification (LLOQ) of the LC-MS assay. This LLOQ was estimated as being 3X greater than the signal generated in aCSF/ascorbic acid alone, or 23 nM. The baseline values in Figure 2 are all below 23 nM and thus these values can be considered to be a background signal. It is only when the dialysis probe is placed in the cocaine-containing solution that cocaine concentrations rise above the LLOQ.

4) Microdialysis probe locations, in fig. 3, should not indicate the tip segment devoid of membrane diffusion (in NAc) since no exchange

is possible.

Reply:
We have modified Fig. 3a such that the segment of the probe where glue was applied—and where no exchange is possible—is now in white, while the active segment of the probe remains in black.

Figure 3 legend

The white boxes at the tips of each probe indicate the segment where glue was applied, and where no exchange is possible.

5) The cocaine dose used in this study (2 mg/kg/infusion) is, contrary to what is written in discussion (p. 19), far from being 'within the range used in drug SA studies'. Actually, most of studies use a 0,175-0,75 mg/kg/infusion range (albeit dose is increased, in some cases, to 1,5).

Reply:
Reviewer # 1 raised a similar concern (Comment # 2). Please see our reply to Reviewer #1. We have also removed the section in the manuscript where the 2 mg/kg cocaine dose was presented as being within the range used in self-administration studies.

6) A weakness affecting this manuscript is that the sole progress it provides to the global knowledge is the possibility to measure both cocaine and other compounds (such as dopamine) simultaneously in freely moving animals. But one could argue that different results other than those presented here would have been very strange, thus limiting the interest for the study. Moreover, many studies have already been published about effects of cocaine infusions rates. One of them, Ferrario et al (2008), also signed by two authors of the present manuscript (Samaha AN, Kennedy RT), described very similar results except that c-fos mRNA was measured but not locomotor activity (and cocaine dosage).

Reply:
Previous studies have shown that varying the speed of i.v. cocaine infusion between 5 and 100 s has large effects on behaviours relevant to addiction (psychomotor sensitization, drug seeking and drug taking), gene expression and heat-producing metabolic activity in the brain. As the Reviewer notes, we have also previously studied effects on striatal dopamine concentrations (Ferrario et al., 2008). However, there are no published accounts of how variation in the speed of i.v. cocaine delivery influences cocaine pharmacokinetics in the brain. We studied this here for the first time. As Reviewer # 1 highlights, this is critical to examine because it is 'highly relevant for our understanding of drug pharmacodynamics and addiction liability'. Measuring brain cocaine concentrations is necessary in order to interpret all work—past and future—where i.v. cocaine infusion rate is manipulated. The new findings in this manuscript suggest that the robust effects of cocaine infusion rate on brain and behaviour reported in previous studies and here (psychomotor activity) are likely not occurring via any large effects on achieved dose. Instead, the observed changes in outcome are likely due to differences in how fast cocaine gets to its target sites in the brain. In other words, beyond how much, how fast drug gets to the brain is likely critical in predicting outcome. Indeed, our findings extend an emerging literature where the pharmacokinetics of drug use are being studied as an active principle in addiction research (Beveridge et al., 2012; Zimmer et al., 2012; Allain et al., 2015).
The Reviewer states that different results would have been strange. Indeed, we expected that different infusion rates would produce different rates of rise of cocaine in the brain. But, without actually measuring brain concentrations of drug, it remained possible that variation in i.v. cocaine infusion rate was producing large differences in achieved dose in the brain—and that this was driving the effects on outcome reported in previous work. Thus, our findings have clear implications for interpreting this work, but also for understanding why drugs, routes and drug formulations that produce a rapid rate of rise of drug levels increase the risk of addiction.
The Reviewer also recognizes that an important contribution of this work is that it provides and validates a very sensitive technique to measure cocaine and other neurochemicals simultaneously, in vivo, in freely moving animals. These advances can inform future work on the link between brain cocaine pharmacokinetics and neurochemical/behavioural response in laboratory animals.

7) Finally, the link between these results and addiction is far from being obvious because nucleus accumbens should have been chosen for microdialysis instead of dorsal striatum. Although the latter is certainly involved in some neural processes related to addiction (formation and maintenance of habits), these processes develop gradually and need repetition of 'cued' instrumental conditioning.

Reply:
We sampled in the dorsal striatum so that the findings could be situated in the context of similar studies with i.v. (Hurd et al., 1988; Hurd & Ungerstedt, 1989; Ferrario et al., 2008) or i.p. cocaine (Kuczenski et al., 1991; Reid et al., 1997). We agree with the Reviewer that it would be worthwhile to measure in other brain regions involved in addiction, including the nucleus accumbens. However, as we summarize in the Discussion section of the manuscript, cocaine kinetics measured in the dorsal striatum likely reflect those in the rest of the brain. We mention in the ms that Hurd et al. (1988) measured cocaine concentrations in both the nucleus accumbens and the caudate-putamen in rats following i.v. cocaine and found no regional differences in extracellular cocaine concentrations. In addition, Berridge et al. (2010) conducted a PET study with nicotine in humans and found that drug pharmacokinetics in individual brain regions do not significantly vary from those in the whole brain. Finally, analysis of post-mortem brains of cocaine users intoxicated at the time of death suggests that cocaine is evenly distributed throughout the brain (Reed & Spiehler, 1985).

8) Neither acute psychomotor effects nor striatal dopamine increase are thought to reflect emotional/subjective cocaine effects such as euphoria (considering that these subjective effects are of prime importance to lead to addiction). So, comparing chronic repeated versus acute administrations of cocaine (for instance with implantation of bilateral cannulae to be used in distinct times) should have been performed in this study in order to perhaps provide new information.

Reply:
The question seems to be, would chronic exposure to cocaine change the brain cocaine and dopamine pharmacokinetics we report here following acute drug exposure? We are very interested in this comparison and it is an important avenue for future work, specifically for dopamine concentrations. This issue is already raised in the manuscript (Discussion, 7th paragraph). However, we would predict that the cocaine pharmacokinetics we describe here would not change with repeated i.v. injection of the drug. Pan et al. (1991) compared acute versus chronic cocaine exposure, and they found that following an intravenous infusion of cocaine, drug concentrations in the nucleus accumbens of naïve (acute) and cocaine-experienced (chronic) rats did not significantly differ. We also note that a single exposure to a psychostimulant drug can produce effects that are relevant to the process of addiction. For instance, a single exposure to cocaine (Guan, Robinson and Becker 1985; Samaha et al., 2002) or d-amphetamine (Robinson, Becker and Presty, 1982) can produce significant psychomotor sensitization in rats. A single d-amphetamine exposure can also evoke psychomotor sensitization in humans (Strakowski and Sax, 1998) and alter spine density on medium spiny neurons of the nucleus accumbens (Kolb, Li, Samaha and Robinson, 2003). This is now discussed in the revised manuscript.

Change to ms:
Discussion, 7th paragraph
Second, the neurobehavioural effects of cocaine that are relevant to addiction come about following chronic exposure to the drug. We do not know how our measurements would change with more extensive drug exposure. Of note, brain and blood concentrations of cocaine do not significantly change following repeated i.v. administration (Pan et al., 1991). However, the ability of i.v. cocaine to inhibit dopamine reuptake can increase with repeated exposure (Brodnik et al., 2017). This being said, studying brain cocaine and dopamine pharmacokinetics after a single cocaine exposure is important. A single exposure to psychostimulant drugs like cocaine or d-amphetamine can produce effects that are relevant to the addiction process, in both laboratory rats and humans. These effects include psychomotor sensitization (Robinson et al., 1982; Lin-Chu et al., 1985; Strakowski & Sax; 1998; Samaha et al., 2002) and changes in spine density on medium spiny neurons of the nucleus accumbens (Kolb et al., 2003).

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2nd Editorial Decision 25 March 2018

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Dear Dr. Samaha,

Your manuscript has been revaluated by one of the original external reviewers and we are pleased to inform you that it will be accepted for publication in EJN pending minor corrections required to comply with EJN guidelines.
1) Remove qualifications from authors list.
2) Correct the list of references so that all of them comply with EJN format.
3) Do not provide p values as inequalities, but provide the exact p value in each case (including for non-significant results).

Additional queries are indicated in the comments below.

Thank you for submitting your work to EJN.

Kind regards,

John Foxe & Paul Bolam
co-Editors in Chief, EJN

Reviews:

Reviewer: 1 Vedran Lovic (University of Calgary, Canada)

Comments to the Author
I am pleased with authors' responses and appropriate modifications made to the manuscript.