EJN European Journal of Neuroscience FENS Federation of European Neuroscience Societies

# "Hot" versus "cold" behavioral-cognitive styles: Motivational-dopaminergic versus cognitive-cholinergic processing of a Pavlovian cocaine cue in sign- and goal-tracking rats

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**Review timeline:**

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| Submission date: | 05 July 2017 |
| Editorial Decision: | 31 August 2017 |
| Revision received: | 08 September 2017 |
| Accepted: | 10 October 2017 |

Editor: Paul Bolam

1st Editorial Decision 31 August 2017

Dear Martin,

First of all, apologies for the long time it has taken us to deal with your manuscript. I guess you understand the problems involved, especially around the summer holiday season! It has now been reviewed by external reviewers as well as by the Editorial team. The reviews collectively indicate that your experiments generated new and important information. However, there are several issues that need to be clarified/resolved before we can consider your manuscript further for publication in EJN.

As you can see the reviewers like your study; they consider it to be relevant, well designed and executed. However, each have raised a series of points that need to be addressed. These can mostly be done by clarification or expansion of the text and constitute 'minor revisions'.

We also note that we need a data statement, and a new policy of the journal is use scatter plots or similar in place of bar charts (see our recent EJN editorial - Rousselet, Foxe and Bolam - http://onlinelibrary.wiley.com/doi/10.1111/ejn.13400/full).

If you are able to respond fully to the points raised, we would be pleased to receive a revision of your paper within 30 days.

Thank you for submitting your work to EJN.

Best wishes,

Paul & John

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

Reviews:

Reviewer: 1 (Mark Walton, University of Oxford, UK)

Comments to the Author
This study examined microdialysis to measure ACh and DA levels in prelimbic cortex during presentation of cues that had or had not been paired with contingent delivery of cocaine in rats categorised as either sign- or goal-tracking. The animals first underwent appetitive Pavlovian conditioning for 5 sessions to a compound lever / light cue to determine whether they predominantly sign tracked (interacted predominantly and more rapidly with the lever rather than the food cup) or goal tracked (vice versa for food cup over the lever). They were next trained with either a paired or unpaired Pavlovian association between a cue light and cocaine for 15 days. Following a washout period, neurochemical levels were measured during a single

extinction session with 4 minute long blocks of cue presentations alternating with 8 minutes blocks of no cues. Sign, but not goal, trackers in the paired group displayed elevated frontal DA levels when they cues were presented, whereas goal, but not sign, trackers in the paired group displayed elevated frontal ACh levels during this period.

This is a nice study, well executed and generally thoroughly presented and analysed. I have a few suggestions to help clarity and for some additional analyses.

1. I didn't find the Introduction particularly informative as, while it summarised the current position regarding ACh, sign/goal tracking and psychological constructs well, there was only a single sentence mention of why it was also worth looking at frontal dopamine levels. I would have liked a bit more reasoning for looking in concert with ACh, even if that reason was basically exploratory. What is already known about medial frontal dopamine levels, Pavlovian conditioning (with either / both cocaine or natural reward) and motivation? Is it believed that ACh and DA work in tandem in that region?

2. I didn't really understand the PCA index as 2 of the 3 measures to be averaged (number of contacts, latency) didn't obviously fall into a -1 to +1 scale. A few more details to explain this to avoid a reader having to go to another paper to understand this would be helpful.

3. I did not fully understand the rationale for going to 5s cue presentations during the microdialysis session when the training had been with a 20s light cue. Given that there is both a change in cue length and outcome (no cocaine) the animals could surmise that the context had changed. Some justification of this and discussion of the potential implications would be helpful.

4. I presume if an animal didn't make a lever or magazine approach, the latency was just recorded as 8s? I couldn't see this stated anywhere.

5. The high levels of approach even on day 1 of cocaine conditioning in the STs paired group, even compared with the STs unpaired, is striking, particularly as levels don't really then change over the subsequent 15 sessions. If performance on day 1 in the 4 groups is broken down trial-by-trial, can these be seen to emerge during the session rather than somehow being there from trial 1?

6. The degrees of freedom for the main effect of phenotype moved about a bit: 1,31 on p13, 1,32 on p14 and 1,34 on p 16. Can the authors check these?

7. Was there a block x phenotype x pairing interaction when examining orienting to the cocaine cues across sessions?

8. While I understand why it was done this way, I think changing the scale on Fig 4c to a max of 0.4 when the comparable plot in Fig 3 is scaled to 1.0 hides the substantial change in responding between session 15 of conditioning and the key extinction day. This should either be changed or the change in scale needs to be explicitly acknowledged in the figure legend or text.

9. While statistically there were no differences between basal levels of ACh and DA, numerically it does look as if there could have been some interesting differences at baseline, with the STs on average having lower DA and paired groups placed in the context having higher ACh levels. Given the low ns for the DA analysis in particular, it would be worth presenting these data in a more transparent way (e.g., box plot with the individuals presented) and/or doing a power calculation to determine whether this is just underpowered.

10. The correlations between neurochemical levels and behaviour are potentially interesting, but I wasn't quite sure what was being depicted in panel e. Are the correlations collapsing across within- and between-subjects variance (so a single rat may contribute multiple individual points that are treated no differently from another rat)? Again, given the low and different ns in the DA groups, it is worth putting in R squared values as well as just "all P>0.18" (p16) as it may be that there is a similar correlation in another group which is just underpowered. Similarly, it would be worth demonstrating that the change in dopamine is specifically related to stimulus-directed behaviour and not just a general increase in locomotor behaviour when the cues are presented. Finally, are there any relationships across individuals between DA and ACh levels?

11. The conditioned reinforcement test is sold as evidence for showing a particularly strong effect in the paired STs. While this is clearly true at some level from the figure, the paired x phenotype isn't actually significant so I'm not sure why a post-hoc test has been run comparing the STs paired v GTs paired if there is only a main effect.

12. It is mentioned in the Discussion that "cortical and subcortical dopaminergic projections act in concert". However, there is, of course, another literature that suggests an inverse relationship (e.g., Pycock et al. 1980; Jackson, Frost, & Moghaddam, 2001). This should be considered.

Minor points:
- Were any rats removed for not having a patent catheter (p7, line 3)?
- Please include details of any analgesics used during and after surgery

Reviewer: 2 (Hans Crombag, University of Sussex, UK)

Comments to the Author
This is an excellent paper, building on previous work by these authors, now demonstrating a double dissociation between the role of prefrontal extracellular ACh and DA levels in mediating cue-evoked behaviors in Sign-tracking (ST) and Goal-tracking (GT) phenotypes. The manuscript is very well written, the methods and data analyses are sound (my expertise in the microdialysis procedures is limited, but this lab has a long reputation in this area so reasonable to assume these are sound), and the results are convincing, interesting and makes a valuable contribution to field. I have only a few minor points:

It seems there are marginal, but orderly, differences in basal levels of DA (as a function of phenotype) and ACh (as a function of conditioning). These are not significant ($p>0.1$) and subsequent measurements are appropriately corrected for, so probably no reason to make much of these in the ms. But it would be interesting to hear what the authors make of is this (if anything)?

In introduction the authors note that the ST phenotype may confer addiction-vulnerability, but what is the evidence for this? As noted by the authors themselves in the conclusion, one might well suggest that GT phenotypes confer vulnerability to relapse by discriminative and/or contextual cues, which are arguably the more likely mechanism (compared to conditioned reinforcement type mechanisms) to relapse in human addicts.

It would be better to refer to the experimental chambers as conditioning chambers, rather the operant test chambers, as no operant procedures (except during testing for CRf) are used.

Please indicate the number of rats used at the start of PCA training, and/or the number of rats that were classified as intermediate.

On p.9 remove the phrase ‘ non-contingent’ - infusions were non-contingent in all conditions.

On p.8 there is some unnecessary repetition of the dialysis probe specifications.

On p.9, line 1. Has repetition of the word block.

Rather than referring to the cue-presentation blocks as C1-C4 or NC, would it not be more straightforward to use CS1-CS4, NCS?

Please specific the number of subjects that were included/excluded based on histological verification of probe placements.

On p.16, the statement at the end of the 1st paragraph should read “… approaches in unpaired STs and paired and unpaired GTs …”.

On the conditioned reinforcement test, there is non-significant 2-way interaction when including the response-contingent unpaired conditions - a more conservative test of CRf. Perhaps and especially because the test was 1-hr in duration, it would be worthwhile to examine the time course of performance, as this would be subject to learning and extinction, and might inform whether the analysis shown in fig. 6 might reasonable be limited to a portion of the test session.

The authors conclude on p.19, that based on their previous studies, GTs are biased towards discriminative cues/occasion setters (i.e., show greater reinstatement effects) and that this bias is hypothesized to be ACh mediated. The authors are certainly welcome to hypothesize this but, may consider that (to my knowledge) the effects of DA manipulations in the ability of DS+/contexts to reinstate drug seeking in GTs (or STs) has not yet been examined.

Authors’ Response 08 September 2017

Dear Paul and John:

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Together with my colleagues we are submitting a revision of our **Research Report** entitled **Hot versus cold behavioral-cognitive styles: Motivational-dopaminergic versus cognitive- cholinergic processing of a Pavlovian cocaine cue in sign- and goal-tracking rats**.

We thank the reviewers for their thoughtful and helpful points. The revisions and our responses to their queries are detailed below.

**Comments by the editors:**

1. *Data accessibility statement.* Response: Such a statement was added.

2. *Scatter plots in place of bar charts.* Response: The prior Figures 5 and 6 were bar graphs. Figure 6 was changed to show scatter plots. However, Fig. 5, that contains 6 different parts, became nearly unreadable when changed to scatter plots, in part because each of the four top parts would show 10 separate populations of data. We also felt that it was important to continue showing the results of *post hoc* multiple comparisons; however, when these comparisons were added to the 10 populations of scatter data per subplot, the entire Figure 5 became nearly indiscernible. Therefore, we decided that Fig. 5 needs to remain in its original version.

Major new text in the manuscript is indicated by red font.

**Reviewer 1:**

1. *Introduction… why it was also worth looking at frontal dopamine levels…What is already known about medial frontal dopamine levels…?* Response: In addition to the Millela paper that was cited in the original version, we added a statement and a reference to a recent, relevant paper (Ellwood et al., 2017). There is a surprising paucity of evidence concerning the role of prefrontal DA in the effects of addictive drug cues. We also added new text to suggest that ventral striatal and prefrontal DA levels may collaborate to support the behavior that identifies STs (p. 4).

2. *I didn't really understand the PCA index…A few more details to explain this to avoid a reader having to go to another paper to understand this would be helpful.* Response: On p. 6, we carefully reviewed our description on how this index was calculated and added more detail (p. 6).

3. *I did not fully understand the rationale for going to 5s cue presentations during the microdialysis session when the training had been with a 20s light cue…Some justification of this and discussion of the potential implications would be helpful.* Response: When exposing an animal to a cue previously paired to a drug (e.g., microdialysis session), we used a shorter cue relative to the conditioning phase for two main reasons: shorter cues result in higher levels of behavioral responding, are associated with less rapid extinction and allow for a greater number of cue presentations within the 4-min dialysis collection blocks. This rationale was added to *Methods* (p. 9).

4. *I presume if an animal didn't make a lever or magazine approach, the latency was just recorded as 8s?* Response: Correct. This detail has been added to *Methods* (p. 6).

5. *The high levels of approach even on day 1 of cocaine conditioning in the STs paired group, even compared with the STs unpaired, is striking, particularly as levels don't really then change over the subsequent 15 sessions. If performance on day 1 in the 4 groups is broken down trial- by-trial, can these be seen to emerge during the session rather than somehow being there from trial 1?* Response: As expected, nearly all animals make approach responses during the first few trials due to the novelty of the cue. It does

appear that there is a trend of increased approach responses in paired STs as early as in the latter half of session/day 1, which is responsible for the observed difference on day 1 approach. This observation is interesting, but it is ultimately more important for the study that the difference between the paired and unpaired groups indicate that approaches are acquired conditioned responses.

6. *The degrees of freedom for the main effect of phenotype moved about a bit…Can the authors check these?* Response: The reviewer queries the denominator dfs across different analyses. As the number of levels of the repeat factors differed across the analyses queried by the reviewer, so do the resulting denominator dfs.

7. *Was there a block x phenotype x pairing interaction when examining orienting to the cocaine cues across sessions?* Response: No such interactions were found (orientating: $F(1,96.62)=0.18$, $P=0.95$; approaches: $F(1,122.77)=0.93$, $P=0.45$).

8. *…I think changing the scale on Fig 4c to a max of 0.4 when the comparable plot in Fig 3 is scaled to 1.0 hides the substantial change in responding between session 15 of conditioning and the key extinction day. This should either be changed or the change in scale needs to be explicitly acknowledged in the figure legend or text.* Response: The rate of cue-directed behavior in the presence of cocaine (Fig. 3) and following 10 days of abstinence and in the absence of cocaine (Fig. 4) are expected to differ markedly. As the reviewer requested, we added a statement to the legend of Fig. 4 to make this point.

9. *While statistically there were no differences between basal levels of ACh and DA, numerically it does look as if there could have been some interesting differences at baseline…* Response: In a different context, we recently published basal absolute prefrontal extracellular ACh and DA levels of STs and GTs and likewise did not find differences (Koshy Cherian et al., 2017). The individual basal values, shown in Cover Letter Figure 1 do not suggest that maintaining the null hypothesis is confounded by the presence of underpowered differences.

10. *The correlations between neurochemical levels and behaviour are potentially interesting, but I wasn't quite sure what was being depicted in panel e. Are the correlations collapsing across within- and between-subjects variance…it is worth putting in R squared values as well as just all P>0.18 (p16) as it may be that there is a similar correlation in another group which is just underpowered. Similarly, it would be worth demonstrating that the change in dopamine is specifically related to stimulus-directed behaviour and not just a general increase in locomotor behavior... Finally, are there any relationships across individuals between DA and ACh levels?* Response: Figure 5e: As stated in Results, this plot shows all data points from all 4 cue presentation periods during which dialysates were collected. Thus, each rat contributed 4 data points (or less if individual dialysates could not have been successfully analyzed). As requested, in Results, we added the R2 values for other groups, and for the correlations with ACh (all are near zero). Locomotor activity: These results are reported on p. 16 (both paired STs and GTs were similarly activated by the cue but only the former produced increases in DA). DA-ACh correlations: None were found.

11. *The conditioned reinforcement test is sold as evidence for showing a particularly strong effect in the paired STs. While this is clearly true at some level from the figure, the paired x phenotype isn't actually significant so I'm not sure why a post-hoc test has been run comparing the STs paired v GTs paired if there is only a main effect.* Response: The reviewer is correct and this legend was corrected and the indication of the phenotype effect was removed.

12. *It is mentioned in the Discussion that cortical and subcortical dopaminergic projections act in concert. However, there is, of course, another literature that suggests an inverse relationship (e.g., Pycock*

*et al. 1980; Jackson, Frost, & Moghaddam, 2001). This should be considered.* Response: Jackson et al. electrically stimulated the PFC and demonstrated effects on DA in the accumbens. Pycock et al. assessed DA receptor binding in the ventral striatum after prefrontal DA lesions. Perhaps with the exception of St. Onge et al. (2012, cited), there seems little evidence showing, in vivo or in the behaving animal, whether or not increases in ventral striatal and PFC DA levels are correlated. The current data suggest that approach behavior to a Pavlovian drug cue is associated with increases in prefrontal DA, paralleling the (phasic) cue-evoked DA responses in the accumbens that were previously shown to be necessary for sign- tracking.

*Minor points: - Were any rats removed for not having a patent catheter (p7, line 3)?* Response: Two rats were removed from the experiment due to loss of catheter patency. Any data collected from these animals were completely discarded from analysis. This detail has been added to Methods (p. 7). - Please include details of any analgesics used during and after surgery. Response: Analgesia details (Carprofen, 5 mg/kg, sc) are detailed in methods and we added that the analgesic was injected at the start of surgery and coverage was maintained for 48 hrs post-surgery. This detail has been added to Methods (p. 7).

**Reviewer 2:**

1. *It seems there are marginal, but orderly, differences in basal levels of DA (as a function of phenotype) and ACh… But it would be interesting to hear what the authors make of is this (if anything)?* Response: See p. reviewer 1, point 9, and the associated Cover Letter Figure 1.

2. *In introduction the authors note that the ST phenotype may confer addiction-vulnerability, but what is the evidence for this? As noted by the authors themselves in the conclusion, one might well suggest that GT phenotypes confer vulnerability to relapse by discriminative and/or contextual cues...* Response: Of course, given our recent paper on this (Pitchers et al., 2017), we fully agree with the reviewer's point. We carefully checked our Introduction and we do not believe that there is a statement that suggest that there is a single, addiction vulnerability- conferring phenotype. The nature of drug cues and prior drug availability/self-administration procedures interact to predict which phenotype exhibit relatively greater drug-seeking behavior (for a recent discussion of this issue see also Kawa, Bentzley & Robinson 2016).

3. *It would be better to refer to the experimental chambers as conditioning chambers, rather than operant test chambers, as no operant procedures (except during testing for CRf) are used.* Response: Agreed, and revised accordingly.

4. *Please indicate the number of rats used at the start of PCA training, and/or the number of rats that were classified as intermediate.* Response: The total number of animals prior to screening was added to *Animals*. As we screened STs and GTs from multiple cohorts over time, we did not accumulate data about intermediates - which were not used in the present experiments. Typically, about a third of the outbred cohort is classified as intermediates.

5. *On p.9 remove the phrase 'non-contingent' - infusions were non-contingent in all conditions.* Response: Done.

6. *On p.8 there is some unnecessary repetition of the dialysis probe specifications.* Response: Fixed.

7. *On p.9, line 1. Has repetition of the word block.* Response: Fixed.

8. *Rather than referring to the cue-presentation blocks as C1-C4 or NC, would it not be more straightforward*

*to use CS1-CS4, NCS?* Response: During non-cue periods no cue was presented, as opposed to the presence of a non-CS; the NC abbreviation properly captures this experimental condition.

9. *Please specify the number of subjects that were included…based on histological verification of probe placements.* Response: This information was added to the legend of Figure 5; based on the histological verification of placements, no set of data was rejected.

10. *On p.16, the statement at the end of the 1st paragraph should read … approaches in unpaired STs and paired and unpaired GTs…* Response: This text was revised (see also Reviewer 1, point 10).

11. *On the conditioned reinforcement test, there is non-significant 2-way interaction…it would be worthwhile to examine the time course of performance…* Response: This test was done *post hoc* to demonstrate that, after abstinence and extinction, the light cue continued to have great motivational value in both phenotypes, thereby suggesting that the ACh-DA differences were specifically associated with the presence and absence of cue-approach behavior. The reviewer makes an interesting point but it is one that is not essential to the interpretation of the results from this *post hoc* test.

12. *The authors conclude on p.19, that based on their previous studies, GTs are biased towards discriminative cues/occasion setters (i.e., show greater reinstatement effects) and that this bias is hypothesized to be ACh mediated. The authors are certainly welcome to hypothesize this but, may consider that (to my knowledge) the effects of DA manipulations in the ability of DS+/contexts to reinstate drug seeking in GTs (or STs) has not yet been examined.* Response: We agree with the reviewer's point and have added this statement on p. 19: In addition to the present evidence in support of this hypothesis, future research would need to demonstrate whether this bias of GTs indeed remains unaffected by manipulations of frontal and mesolimbic dopaminergic activity.

We again wish to thank the reviewers for their highly productive comments and we hope that you will agree that the revised paper has much improved and is now ready for publication in EJN.

Sincerely, Martin.

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**Cover letter Figure 1**