The *in vivo* effects of solid drug nanoparticle and conventional efavirenz on anixiogenesis in rodents

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Introduction

- Efavirenz (EFV) has variable bioavailability and induces neurocognitive side effects, including anxiety.
- Recently we reported an EFV solid drug nanoparticle (SDN) formulation with pharmacological benefits[1].
- Here we investigated neurocognitive disturbances in rats using the elevated plus maze (EPM) following oral administration of a pre clinical conventional or solid drug nanoparticle (SDN) formulation of EFV.
- The EPM has been previously validated as a method to assess anxiety in rats and mice in response to exposure to EFV[2+3].

Methods

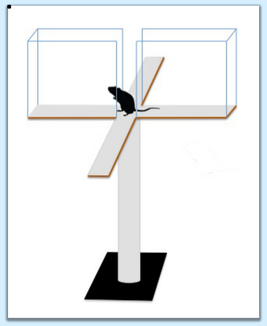


Fig 1 diagram showing the elevated plus maze

Behavioral Measure	Description
Open arm entrances	An entrance to open arm is counted as 4 paws placed on the open arm
Closed arm entrances	An entrance to closed arm is counted as 4 paws placed on the closed arm
Time in open arm	The time (expressed as a percentage of total time on the maze) spent in the open arms
Time in closed arm	The time (expressed as a percentage of total time on the maze) spent in the closed arms
Time on central platform	The time (expressed as a percentage of total time on the maze) spent on the central platform
Central platform latency	Time taken to initially move from central square
Risk assessment	The number of occurrences of behavior indicating assessment of the environment for potential risk factors (stretch attend postures, head dips and rearing)
Sedation	The total time spent stationary (grooming and non exploratory behavior)

Table 1 summary of the spatial and ethological measures recorded during exposure to the elevated plus maze

- Male Wistar rats were administered EFV (10mg/kg, 0.5% methylcellulose), SDN (10mg/kg) or vehicle control by oral gavage once daily for 5 weeks.
- Rats were placed in the elevated plus maze for 5 minutes weekly.
- Behaviour was videotaped and analysed using Ethowatcher software.
- Data were collected on number of entrances into the open and closed arms and time spent in the open arm, closed arm and central square (expressed as percentage of total time on EPM) and central platform latency.
- Additionally, ethological measures were recorded (Table 1) and used to generate composite measures of risk assessment (a combined total of occurrences of risk assessing behaviour) and sedation (a total of the duration of all non exploratory behaviour).
- Statistical analysis was performed by Mann-Whitney U test and significance was defined as P <0.05. All data are given as median [IQR].

Results

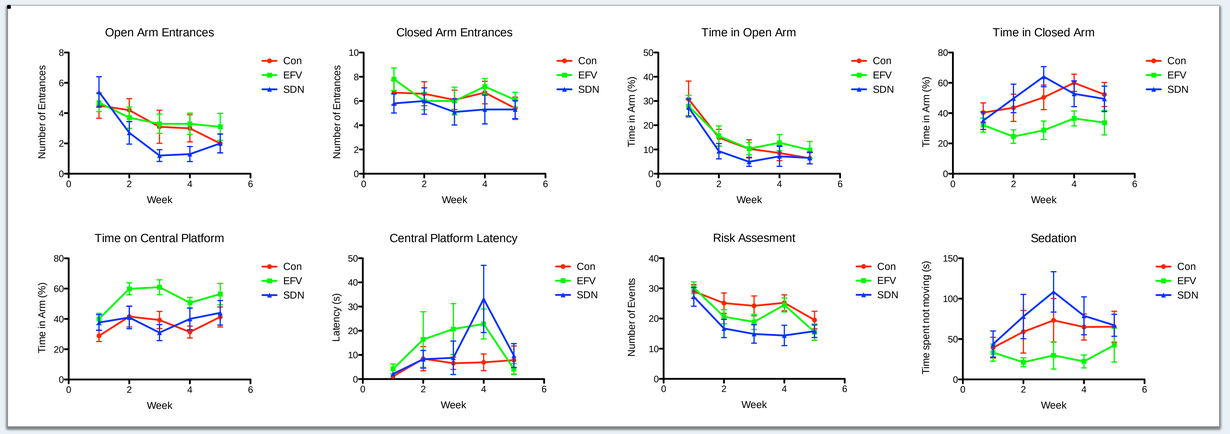


Fig 2 graphs show the behavioural measures plotted against time from the elevated plus maze for control (Con), conventional efavirenz (EFV) and solid drug nanoparticle efavirenz (SDN). The graphs display time plotted against spatial measurements open arm entrances, closed arm entrances, time in open arm, time in closed arm, time on central platform and central platform latency. Also shown are the composite measures for risk assessment and sedation. Data points represent median with interquartile range.

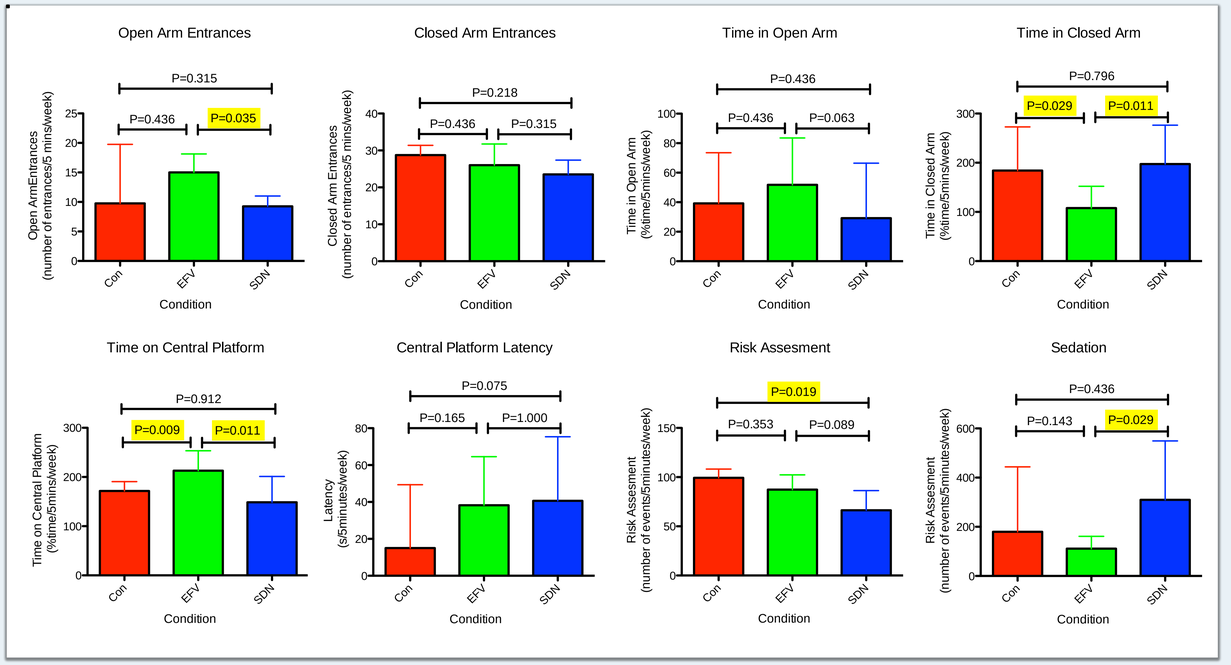


Fig 3 shows the area under the curve for each of the measurements displayed in fig 2. Data represent mean with interquartile range. Also displayed are the results of the Mann-Whitney U test and significance was defined as P <0.05 (significant results are highlighted in yellow)

- No differences in behaviour were observed in week 1.
- In week 2, the percentage of time spent on the central platform was significantly increased in the EFV group (60% [51-65%]) compared to SDN (44% [25-50%]) and control (47% [34-57%] with P = 0.03 and P = 0.91 for EFV and SDN versus control, respectively.
- In week 3 differences in time spent on the central platform were again observed (EFV 65% [48-71%], SDN 31% [25-42%], control 46% [28-52%], with P = 0.01 and P = 0.28 for EFV and SDN versus control, respectively. Significant differences in the time spent in the closed arm were also observed (EFV 21% [13-46%], SDN 66% [47-75%], control 43% [33-71%]) with P = 0.005 and P = 0.631 for EFV and SDN versus control, respectively.
- Similarly in week 4, time spent by the EFV group on the closed arms was lower (EFV 34% [28-42%], control 61% [IQR 46-72%]; P = 0.005) and time on the central platform was higher (EFV 51% [46-57], control 30%, [26-42%]; P = 0.001).
- No statistically significant differences in behaviour between any groups were observed in week 5.
- In addition to examining behaviour weekly, data was plotted against time to compare behaviour across duration of the experiment (Fig 2).
- The AUC of the EFV group showed significant differences in spatial distribution when compared to the control group, time in closed arm (Con 184.2 [130.3-272.8], EFV 107.9 [87.74-152.1]) and time on central platform (Con 171.7 [91.9-190.7], EFV 212.9 [188.3-253.3]) (Fig 3).
- The AUC of the SDN group did not significantly differ from the control group except for a reduction in risk assessment (Con 99.25 [84.0-108.1], SDN 87.25 [69.6-102.3]) (Fig 3).

Conclusions

- Our experiment did not fully replicate EFV anxiogenic effects previously reported using the EPM[2+3], but did show clear behavioural effects indicative of CNS activity.
- Notably, a tendency of EFV to increase time spent on the central platform may be indicative of anxiogenesis[4].
- By contrast, the SDN did not consistently affect behaviour in a manner that would indicate anxiogenic activity.
- We interpret these data as indicating the SDN may have reduced potential to induce neurocognitive disturbance, either acutely or after long-term administration.

References

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