Neural mechanisms underlying sensitisation to drugs of abuse: clues to the mechanisms of dependence?

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Background

Behavioural sensitisation, measured as the progressive, long lasting increase in locomotor activity, occurs with repeated administration of psychostimulants drugs such as amphetamine and nicotine.

It is thought to result from neuroadaptive changes occurring in the mesolimbic dopaminergic pathway projecting from ventral tegmental area (VTA) to nucleus accumbens (NAc), which contribute to addiction.

Several studies have demonstrated that the acute action of psychostimulant drugs in VTA is an important cellular event for initiation of behavioural sensitisation while behavioural expression that occurs after repeated drugs exposure is mediated in the neural circuitry in the NAc [Kalivas & Stewart, 1991, *Brain Res. Rev.* **16**, 223-244]

The prolonged time course of behavioural sensitization in psychostimulant dependence has raised the question of whether long-term drug-induced alteration in gene expression, including immediate early genes (IEGs) such as Arc and *c-fos* and the transcription regulator (TR) Mecp2, plays a critical role in sensitisation [Nestler, 1992. *J. Neuroscience*, **12**, 2439-2450].

Thus, the mesolimbic pathway is believed to influence behavioural sensitisation and may display increased IEG expression after psychostimulant administration.

Study Aims

To investigate the cellular mechanisms underlying psychomotor sensitisation to amphetamine and nicotine, and cross-sensitisation between the two drugs, by measuring IEG and TR activation.

Methods

- Male Lister Hooded rats (300 ± 50g) were randomly assigned to one of seven experimental groups (table 1), and handled daily for 3 days.
- Drug sensitisation was achieved by giving each animal a daily injection of the relevant drug, or saline vehicle (table 1), in a volume of 1 ml/kg; i.p..
- Each animal was placed immediately in an activity box (24 x 46 cm) for 60 min, and their activity was monitored using AnyMaze software (San Diego Instruments, California, USA).

Group	Pretreatment	Challenge
1	Saline	Saline
2	Saline	Amphetamine
3 *	Amphetamine	Amphetamine
4 †	Nicotine	Amphetamine
5	Saline	Nicotine
6 *	Amphetamine	Nicotine
7 †	Nicotine	Nicotine

Drug/vehicle doses used in pre-treatment and challenge

Saline : *1ml/kg, i.p.*
Amphetamine: *1 mg/kg, i.p.*
Nicotine: *0.6 mg/kg, i.p.*

** = sensitisation groups*
† = cross-sensitisation groups

- After 10 days withdrawal (drug free) rats were challenged with amphetamine or nicotine and activity was recorded for 60 min as above.
- Two hours later animals were culled, and brains were fixed by transcardiac perfusion with 4% para-formaldehyde in 0.9% saline (PFA).
- Brains were removed and post fixed in PFA for 24 h.
- Prior to sectioning, brains were rinsed with 0.1M PBS (pH =7.4) and cryo-protected in sucrose (15-30%), then frozen in isopentane and stored at -80 C.
- Coronal sections (30 µm) were cut using a cryostat (Thermo Scientific NX50, Germany) and sections including NAc were selected.
- For immuno-staining free-floating sections were:
  - Blocked with 3 % H_2O_2 for 10 min .
  - Incubated in PBS with 5% normal goat serum (NGS) for 30 min
  - Incubated with the relevant primary antibodies and 1% NGS with PBS/1% Tx at 4 C for two nights under gentle shaking.
  - Incubated with biotinylated goat anti-rabbit IgG (1:500; Vector Laboratories) with 1% NGS for 60 min
  - Incubated with avidin-biotin complex (1:200; ABC; Elite Kit, Vector Laboratories) for 60 min.
  - Immunoreactivity was revealed by treating with nickel-enhanced 3'3'diaminobenzidine (DAB-Ni) with 0.01% H_2O_2 for 5 min.
  - Dehydrated and mounted.
- Sections were washed with 0.1 M PBS between each stage
- Primary antibodies used:
  - c-fos: polyclonal rabbit anti-(c)-fos (1:2000; Santa Cruz Biotech., CA, USA);
  - Arc: C-Terminal rabbit anti-ARC (1:2000; Sigma-Aldrich, Germany)
  - Mecp2: anti - Mecp2 (1:2000; Millipore EMD)

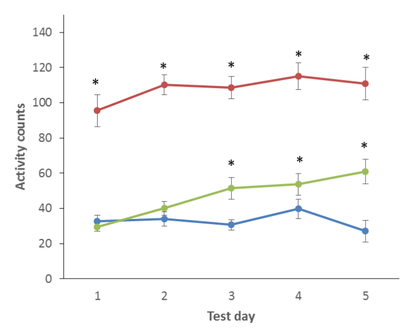
Results : Behavioural sensitisation

Animals showed behavioural sensitisation with both amphetamine and nicotine, although the two drugs showed different time courses.

Amphetamine treatment caused hyperlocomotion on all pre-treatment days (fig 1), and a sensitised response on challenge day (fig 2a)

Nicotine treatment initially had no effect on activity, but hyperlocomotion gradually established with repeated pre-treatment (fig 1). A sensitised response was also seen on challenge day (fig 2b)

There was no behavioural cross sensitisation between the two drugs with either amphetamine (fig 2a) or nicotine (fig 2b) challenge.

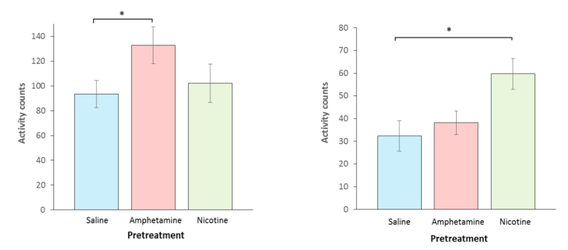


***ig. 1: Locomotor activity in pre-treatment** Mean (± SEM) activity counts during five days of re-treatment with amphetamine (red) or icotine (green), compared to animal treated vith saline vehicle (blue).*

** p < .05; significant difference from saline pre-treated animals: Fisher's protected LSD. (n = 10 – 12 per group)*

Fig 2: Effect of challenge dose of (a) amphetamine or (b) nicotine after pre-treatment with saline, amphetamine or nicotine

** p < .05; Significant difference from saline pretreated animals: Fisher's protected LSD. (n = 5 – 6 per group)*



Results : Immunohistochemistry

Although there were some changes in NAc shell (fig. 3a), the main changes in IEGs were in NAc core (fig. 3b): there was evidence of sensitised increase in Arc, *c-fos* and Mecp2 after nicotine challenge.

There was evidence of cross-sensitisation between amphetamine pre-treatment and nicotine challenge (AMP-NIC: red bar) in NAc core (fig 3b).

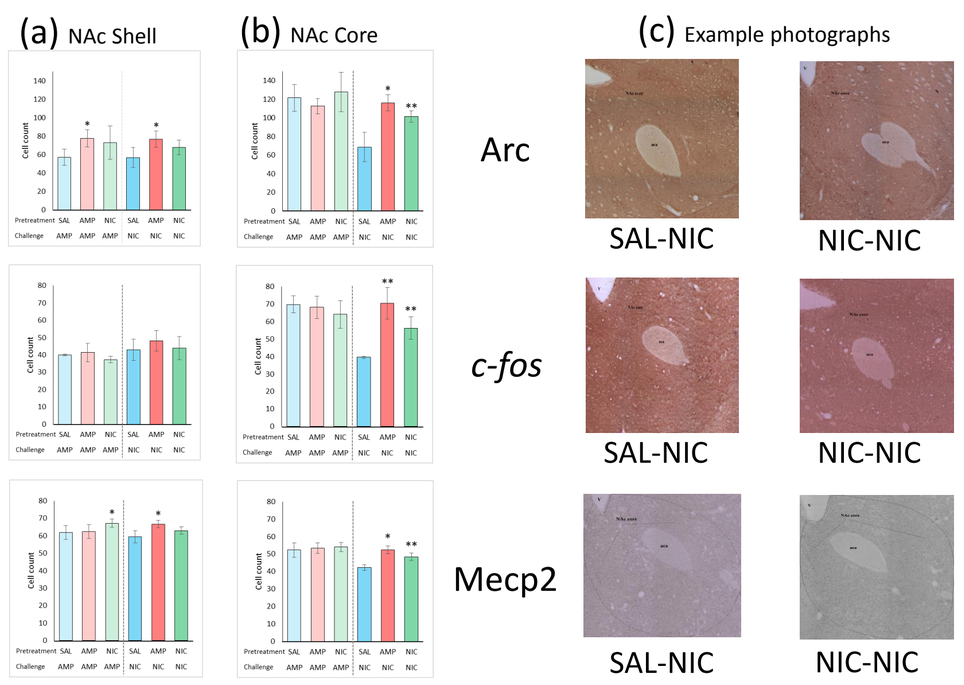


Fig 3: Immunohistochemical analysis of immediate early genes Arc, c-fos and Mecp2 in (a) NAc shell and (b) NAc core slices after challenge with amphetamine (AMP) or nicotine (NIC) following pre-treatment with saline (SAL), amphetamine or nicotine. (c) Example photographs showing increased staining in NAc slices from nicotine challenged animals pre-treated with saline (SAL-NIC) or nicotine (NIC-NIC).

** p < .05; ** p < .01 Significant difference from saline pretreated animals: Fisher's protected LSD.*

Conclusion

Behavioural sensitisation to nicotine, but not amphetamine was accompanied by increased IEG activity in NAc core.

Although there was no behavioural cross sensitisation, there was cross sensitisation of EIG activity in NAc core.

These results provide evidence of epigenetic changes in neuronal function in NAc core during psychostimulant sensitisation

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