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Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: Attenuation after chronic fluoxetine treatment

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ABSTRACT

Motor stereotypies, described as repetitive, topographically invariant and seemingly purposeless behaviours, are common to several developmental and neuropsychiatric disorders. While drug induced stereotypy has been extensively studied, the neurobiology of spontaneous stereotypy is poorly understood. Deer mice present with naturalistic stereotypic behaviours that are selectively suppressed by fluoxetine. We studied basal cyclic adenosine monophosphate (cAMP) levels and phosphodiesterase (PDE) type 4 activity in prefrontal cortex and striatum of high, low and non-stereotypic deer mice, as well as response in high stereotypic mice to chronic fluoxetine treatment (20 mg/kg/day×21 days intraperitoneally). Cortical cAMP levels were associated with stereotypic behaviour, being significantly elevated in low and high stereotypic mice compared to non-stereotypic animals, with a similar trend in the striatum. In both brain regions, there was a significant inverse correlation between PDE4 activity and stereotypic behaviour. In the prefrontal cortex, PDE4 activity was significantly reduced in both low and high stereotypic mice compared to their non-stereotypic controls, while in the striatum, only high stereotypic mice showed a significant reduction in PDE4 activity. Fluoxetine significantly attenuated stereotypies in high stereotypic animals, together with a reduction in cortical cAMP levels and PDE4 activity, without noteworthy effects in the striatum. Spontaneous stereotypy in deer mice is thus characterized by raised cAMP and reduced PDE4 enzyme activity, particularly in the prefrontal cortex, and is modified by chronic treatment with fluoxetine.

1. Introduction

Restricted, repetitive behaviours are common behavioural phenotypes of a number of neuropsychiatric and related neurodevelopmental disorders, including obsessive compulsive disorder (OCD), OCD-spectrum disorders (APA, 2000; Stein, 2000), autism (Turner, 1999) and schizophrenia (Tibbo and Warneke, 1999). The neurobiological underpinnings of stereotypy are largely unknown, although several lines of evidence support the role of aberrant cortico-striatal feedback circuits (Ernst and Smelik, 1966; Bedingfield et al., 1997; Korff and Harvey, 2006; Lewis et al., 2007), and each of which emphasises the role of dopamine and glutamate. An animal model that presents with naturalistic spontaneous stereotypy that does not require induction by pharmacological or other means, would provide a valuable tool with which to explore the underlying neurobiology of stereotypy (Korff and Harvey, 2006; Lewis et al., 2007).

The deer mouse (*Peromyscus maniculatus bairdii*) presents with a unique array of unvaring and functionless locomotoric behaviours that are repetitive in nature (Presti and Lewis, 2005; Korff et al., 2008). Moreover, these stereotypies occur spontaneously when housed under standard housing conditions (Powell et al., 1999), while recent evidence has established that these abnormal repetitive behaviours are mediated by disturbances in cortico-striatal thalamo-cortical (CSTC) circuits (Lewis et al., 2007; Presti and Lewis, 2005). We demonstrated the involvement of 5-HT_{1A}, 5-HT_2 and D_2 receptor signalling in these behaviours, as well as inhibition by a serotonin (5-HT) reuptake inhibitor (SRI) but not a noradrenergic reuptake inhibitor (Korff et al., 2008). This not only confirms the involvement of the dopamine and serotonin systems in the expression of these behaviours, but also demonstrates the predictive validity of the deer mouse model for OCD, a neuropsychiatric disorder characterized by abnormal repetitive behaviours. Thus, although OCD symptoms may be goal directed while stereotypies lack a goal or function (Garner, 2005), similar neurobiological mechanisms may be involved in both abnormal repetitive behaviours in animals and in OCD.

The clinical efficacy of SRI's in OCD has been linked to the gradual down-regulation of pre-synaptic 5-$HT_{1A/B/D}$ receptors (Bergqvist et al., 1999; El Mansari and Blier, 2006), which couple negatively to adenylate cyclase-cyclic adenosine monophosphate (cAMP) signalling (Barnes and Sharp, 1999). Several studies have demonstrated adaptations at

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various levels of the cAMP signal transduction cascade in response to antidepressant treatment, including increased G-protein-adenylate cyclase coupling (Ozawa and Rasenick, 1991), increased expression/activity of the cAMP response element binding protein (CREB) (Nibuya et al., 1996), and increased phosphodiesterase 4 (PDE4) expression (Takahashi et al., 1999). Both cAMP and one of its principle metabolising enzymes, PDE4, are considered important therapeutic targets for neuropsychiatric disorders (Hudson et al., 1993). Although some evidence has supported a role for cAMP-dependent signalling in disorders involving abnormal repetitive behaviours such as OCD (Perez et al., 2000; Marazziti et al., 2001), to our knowledge this has not been studied in animal stereotypy. Analysing the regional brain neurobiology of perseverative behaviours, especially of the basal ganglia and cortico-striatal loop, will shed more light on the mechanisms underlying spontaneous stereotypy. We therefore chose to investigate cortico-striatal cAMP levels and PDE4 activity during varying states of spontaneous stereotypy in deer mice, as well as response of these parameters to chronic fluoxetine administration in high stereotypic animals.

Since earlier studies (Korff et al., 2008) demonstrated reduced stereotypy in high stereotypic deer mice following administration of a non-selective $5HT_{1A}$ agonist, we predicted that increased stereotypy (as in high stereotypic deer mice) would be characterized by reduced $5HT_{1A}$-Gi dependent adenylate cyclase coupling and increased cAMP. If the latter changes are mediated by altered breakdown of cAMP as opposed to altered synthesis, an associated decrease in PDE4 activity will be evident. Furthermore, we also hypothesized that any changes in cAMP-PDE4 signalling would demonstrate regional differences within the CSTC circuit. Finally, both stereotypic behaviour and abnormal brain neurochemistry would be modified following chronic administration of fluoxetine.

2. Methods

2.1. Materials

Cyclic AMP [^{3}H] radioimmunoassay kits were obtained from Amersham Biosciences (Johannesburg, South Africa). Anion exchange resin (AG 1-X8, 100–200 mesh, formate form), was purchased from Bio-Rad (Johannesburg, South Africa). BCA reagent for protein determination was acquired from Pierce (Johannesburg, South Africa). Scintillation fluid (Ultima gold XR fluid) was obtained from PerkinElmer (Johannesburg, South Africa). Fluoxetine (FLX) was a kind donation from Aspen Pharmacare (Port Elizabeth, South Africa), while rolipram and all other chemicals were obtained from Sigma-Aldrich Chemicals (Johannesburg, South Africa).

2.2. Animals

Stereotypic behaviour in the deer mouse becomes evident early in life, being most notable at about 20 days of age and becoming well developed by 30 days of age. Deer mice develop high rates of persistent, spontaneously emitted stereotypy which continue at stable levels when housed under standard laboratory conditions (Powell et al., 1999). Typical topographies include somersaulting, jumping and pattern running, which in various studies have been confirmed as being repetitive and stereotypic in nature (Powell et al., 1999; Presti and Lewis, 2005; Korff et al., 2008). Indeed, our earlier studies have compared high stereotypic deer mice to C57Bl mice and confirmed that the above behaviours are repetitive, unvarying and functionless in nature (Korff et al., 2008). Computerised stereotypy scores were also corroborated with visual monitoring. Animals of 8 weeks (56 days) or older were chosen in order to reduce the degree of variability in the rate of stereotypy development between animals. Deer mice were obtained from the *Pyromyscus* Genetic Stock Centre, University of South Carolina, Columbia, USA, with animals of both sexes used as they display equal amounts of stereotypy (Powell et al., 1999). Deer mice were housed in standard (40 cm × 25 cm × 20 cm) laboratory cage, four mice per enclosure to ensure freedom of movement. The animals were allowed free access to food and water under controlled environmental conditions (24 °C; 12-h light/dark cycle, lights off at 18:00 h). The study was conducted in accordance with the guidelines stipulated by the Ethics Committee for use of experimental animals at North-West University (Ethics Approval number: 04D09).

2.3. Behavioural testing

Behavioural separation of animals was performed as described previously (Korff et al., 2008), using a computerised animal activity monitor (Digiscan Animal Activity Monitor (DAAM); AccuScan Instruments, Columbus, Ohio, USA). The DAAM cages included a drop-down grid-roof to simulate the home cage environment and also to facilitate somersaulting behaviours. This methodology has been well described in its ability to isolate and quantify the three principle stereotypic behaviors displayed by deer mice, viz. somersaulting, jumping and pattern running (Presti and Lewis, 2005; Korff et al., 2008). As per our earlier work (see Korff et al., 2008 for detailed description), deer mice of either sex were separated according to their behavioural phenotype. Briefly, animals were assembled into three groups according to stereotypic score (i.e. counts per hour or Cph), namely high stereotypic behaviour (Cph > 2000, HSB), low stereotypic behaviour (Cph 1000–2000, LSB) and non-stereotypic (Cph < 1000, NS). Cut-off points were chosen according to a previous study (Korff et al., 2008) (see legends to figures for *n* values). All behavioural assessments were performed during the animal's active cycle, i.e. at night. Since we were primarily interested in whether heightened stereotypy and its associated biochemical correlates were sensitive to 5-HT manipulation, cortico-striatal cAMP-PDE4 changes following chronic saline or FLX treatment were studied in only HSB mice. This was done to assess whether any observed changes in CSTC neurochemistry could be related to concomitant behavioural changes induced by the drug. In an earlier study, we demonstrated that chronic FLX treatment significantly suppresses spontaneous stereotypy in the deer mouse without noteworthy effects on measures of general locomotor activity (Korff et al., 2008). For the drug treatment cohort, HSB mice were screened on three occasions for basal stereotypy and again at the end of the 3 week treatment period (see legends to figures for *n* values). Animals were sacrificed immediately thereafter and brain tissue removed for analysis as described below. In all cases, animals were sacrificed at the end of their active cycle between 07h00–09h00 in order to avoid possible variations in cAMP levels and PDE4 activity due to circadian rhythms.

2.4. Drugs

FLX was dissolved in 0.9% saline and administered intra-peritoneal to a maximum volume of 300 µl per animal (0.012 ml/g). All mice used in the pharmacological study were prior drug and treatment naïve. FLX was administered at a dose of 20 mg/kg for a period of 21 days (Korff et al., 2008). No-drug controls received an equivalent volume of saline.

2.5. Preparation of tissue

Mice were killed by decapitation and the brain rapidly removed and dissected on an ice-cooled dissection slab. After removing the olfactory bulb, the prefrontal cortex was defined according to Guldin et al. (1981). The medial orbital and medial frontal lobe (pre-limbic and infra-limbic) were dissected and fixed in liquid nitrogen. For the striatum dissection, the brain was placed dorsal side up and,

after splitting of the two cerebral hemispheres, the striatum was dissected with the external walls of the lateral ventricles as internal limits and the corpus callosum as external limits. The striatum is a well-defined structure resembling an orange wedge in texture and shape, and the characteristic striated appearance aids identification. These wedges were loosened from the cortical tissue and freed from the midbrain and immediately frozen in liquid nitrogen. Both sets of tissue were then stored at −80 °C until undergoing the extraction procedures and analysis.

2.5.1. cAMP

Immediately prior to cAMP determination, brain tissue was thawed on ice and deproteinised and processed according to the manufacturer instructions for radioimmunoassay of tissue cAMP described below. Briefly, the whole striata or frontal cortices for each animal was used for analysis of cAMP. All available samples where pooled for each stereotypic group (see legends to figures for *n* values). Pooled samples were thawed and suspended in 2 ml ethanol, thoroughly mixed and left to stand for 5 min at room temperature. Tissue samples were homogenized (10–12 strokes) with a Teflon homogenizer. A small aliquot of sample (150 µl) was saved for protein determination and the remaining sample centrifuged (3 min at 2000 ×*g*). Supernatant was transferred to a new tube and used for cAMP determination.

2.5.2. PDE4

For the PDE4 activity assay, thawed tissue from individual mice was homogenized in sample buffer containing 20 mM Tris–Cl (pH 7.2), 1 mM EDTA and 250 mM sucrose using a Teflon homogenizer (15 strokes, 4 °C). The homogenates were sonicated on ice (30 s/ml) and the pH adjusted to 6.0 with 1 M acetic acid. A small aliquot of sample (150 µl) was saved for protein determination and the remaining samples centrifuged at 1000 ×*g* for 15 min. PDE4 activity was subsequently determined in the supernatant.

2.6. Determination of cAMP accumulation and PDE4 activity

The Amersham Biosciences cyclic AMP [^{3}H] assay kit was used to determine cAMP levels as per the manufacture's protocol. PDE4 activity was assayed using a modified method of that described by Torphy and Cielinski (1990). PDE4 activity is determined by subtracting non-PDE4 activity (samples in the presence of the selective PDE4 inhibitor, rolipram) from the total PDE activity (samples without rolipram). Briefly, following sample preparation the reaction was initiated by adding pre-warmed enzyme (10 µg protein) into standard reaction mixture containing (final concentrations): 40 mM Tris–Cl (pH 8.0), 10 mM $MgCl_2$, 1.25 mM *B*-mercaptoethanol, 0.75 mg/ml bovine serum albumin, 100 000 counts per minute (c.p.m.) [^{3}H] cAMP, and 2.5 µM authentic cAMP. The final reaction mixture also included or excluded 100 µM rolipram, as required. Following incubation at 34 °C for 10 min, the reaction was terminated by the addition of an equal volume of ice-cold stop buffer (40 mM Tris–Cl, pH 7.4, and 10 mM EDTA). Samples were then boiled for 2 min at 94 °C and transferred to an ice bath for 3 min. An excess amount of nucleotidases (100 µl of a 100 mg/ml stock solution) in the form of snake venom (*Crotalus atrox*) was added to the samples, followed by incubation for 15 min at 34 °C. The reaction was stopped by adding 2 ml ice-cold ethanol. The entire reaction mixture was then subjected to anion-exchange chromatography by adding anion resin to the test tube. Tubes were briefly vortexed and incubated for 10 min at room temperature followed by centrifugation for 3 min at 500 ×*g*. Supernatant (1 ml) was dispersed in triplicate to scintillation vials and 4 ml liquid scintillation fluid added to each tube. Radioactivity was measured by scintillation spectroscopy (TRI-CARB 2100TR liquid scintillation analyser), with PDE4 activity expressed as pmol/min/mg protein.

2.7. Statistical analysis

Graphpad Software was used for the statistical analysis of all data, while graphics were produced using GraphPad Prism version 4.00 for Windows (Graphpad Software, San Diego, USA). For the cAMP and PDE4 activity analysis in the various stereotypic populations, group means were analysed using one-way analysis of variance (ANOVA) followed by Tukey *post hoc* tests. Analysis of the pre-and post-treatment response to FLX or saline was analysed with the two-tailed Student's *T*-test. Results are presented as mean ± S.E.M. A probability level of 95% was used to determine statistical significance ($p<0.05$). The association between stereotypy and cAMP, and between stereotypy and PDE4 activity, was tested using Spearman's rank correlations as implemented on SPSS 16.

3. Results

3.1. cAMP levels in the prefrontal cortex and striatum of deer mice

Basal cAMP levels in the brain of low- and high stereotypic deer mice were compared to that of non-stereotypic deer mice (control). One-way ANOVA revealed significant differences across the groups for cAMP in the prefrontal cortex [$F(2;54)=6.42$; $p=0.003$] (Fig. 1A) but not in the striatum [$F(2;31)=1.50$; $p=0.24$] (Fig. 2A). Post hoc testing indicated that basal cAMP levels of HSB and LSB mice in the prefrontal cortex were significantly higher than that of NS mice

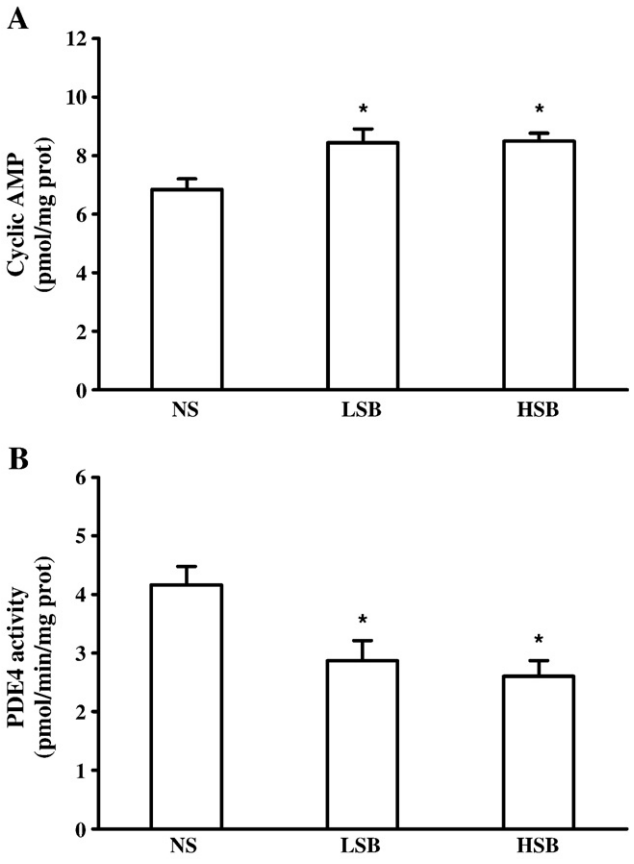

Fig. 1. Cortical cAMP levels (A) and PDE4 enzyme activity (B) in low stereotypic (LSB) and high stereotypic (HSB) deer mice compared to non-stereotypic (NS) mice. Prefrontal cortex cAMP levels were determined for HSB ($n=15$), LSB ($n=18$) and NS ($n=26$) mice, as indicated. Additionally, PDE4 enzyme activities were determined for HSB ($n=9$), LSB ($n=10$) and NS ($n=6$) mice. Significant differences versus control NS mice are indicated by an asterisk (one-way ANOVA followed by the Tukey test; $*p<0.05$). Data are expressed as mean ± S.E.M.

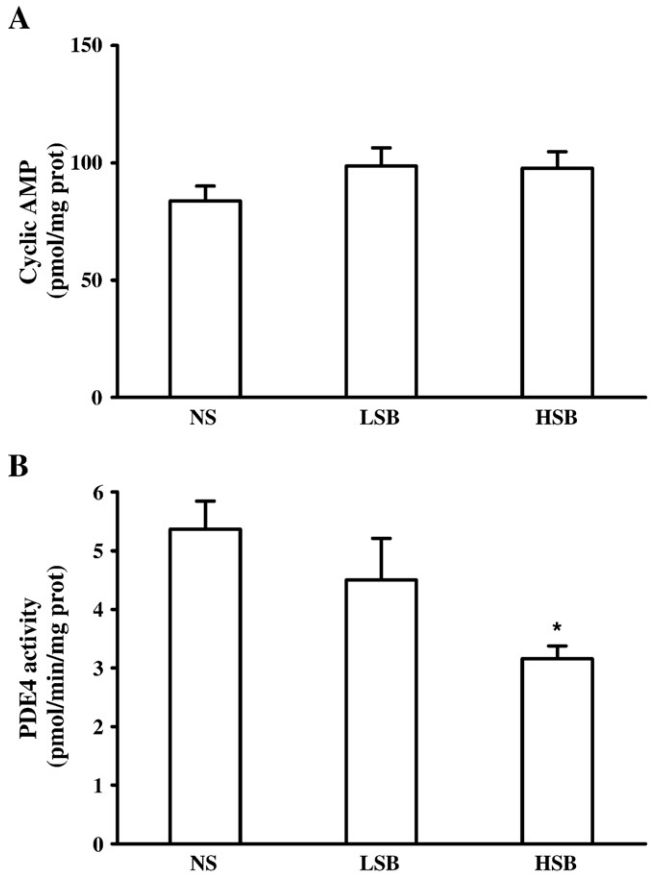

Fig. 2. Striatal cAMP levels (A) and PDE4 enzyme activity (B) in low stereotypic (LSB) and high stereotypic (HSB) deer mice compared to non-stereotypic (NS) mice. Striatal cAMP levels were determined for HSB ($n=8$), LSB ($n=8$) and NS ($n=18$) mice, as indicated. Additionally, PDE4 enzyme activities were determined for HSB ($n=7$), LSB ($n=7$) and NS ($n=10$) mice. Significant differences versus control NS mice are indicated by an asterisk (one-way ANOVA followed by the Tukey test; *$p<0.05$). Data are expressed as mean ± S.E.M.

($p<0.05$; Fig. 1A), although HSB and LSB mice were not significantly different from one another (Fig. 1A). Stereotypic behaviour was positively associated with cAMP levels in striatum ($r=0.24$, $p>0.05$) and cortex ($r=.39$, $p>0.05$), although this association did not reach statistical significant.

3.2. PDE4 activity in the prefrontal cortex and striatum of deer mice

Basal PDE4 activity of low- and high stereotypic deer mice were compared to that of non-stereotypic deer mice (control). One-way ANOVA revealed significant differences across stereotypic groups for PDE4 activity in both the prefrontal cortex [$F(2,22)=5.47$; $p=0.01$] (Fig. 1B) and striatum [$F(2,21)=4.91$; $p=0.02$] (Fig. 2B). Subsequent post hoc testing indicated that basal PDE4 activity in the prefrontal cortex in HSB and LSB mice was significantly lower than that in NS mice ($p<0.05$; Fig. 1B), with that in HSB and LSB mice not differing significantly from one another (Fig. 1B). In the striatum, PDE4 activity was significantly lower in HSB mice compared to NS mice ($p<0.05$; Fig. 2B). While that of LSB mice was numerically lower than NS mice and higher than that of HSB mice, these differences did not reach statistical significance (Fig. 2B). Stereotypies correlated inversely with PDE4 activity in the striatum ($r=-0.54$, $p=0.01$) and cortex ($r=-0.51$, $p=0.01$). These negative correlations reached statistical significance and may be deemed of practical importance (see Ellis and Steyn, 2003).

3.3. Effect of FLX on cortico-striatal cAMP levels and PDE4 activity

To explore the effect of chronic SRI treatment on cortico-striatal cAMP-PDE4 signalling, high stereotypic (HSB) deer mice received chronic treatment with either FLX (20 mg/kg/day ip) or saline (control) for 21 days. In the prefrontal cortex, FLX evoked a significant reduction in both cAMP levels ($p<0.05$; Fig. 3A) and PDE4 activity ($p<0.05$; Fig. 3B) compared to saline-treated animals. Striatal cAMP levels and PDE4 activity, however, were unaffected by FLX treatment (Fig. 4A and B), although there was a trend towards reduced PDE4 activity that did not reach statistical significance.

3.4. Effect of FLX on the expression of stereotypic behaviours

High stereotypic (HSB) deer mice received chronic treatment with either FLX (20 mg/kg/day ip) or saline (control) for 21 days. FLX treatment evoked a significant reduction in stereotypic behaviours on day 21 compared to baseline levels on day 1 of treatment ($p=0.04$; Fig. 5), while saline-treated animals showed no difference with respect to pre-and post-treatment levels (Fig. 5).

4. Discussion

This paper has demonstrated that prefrontal cortical cAMP levels are significantly elevated in low and high stereotypic deer mice compared to non-stereotypic animals, with a similar trend observed in the

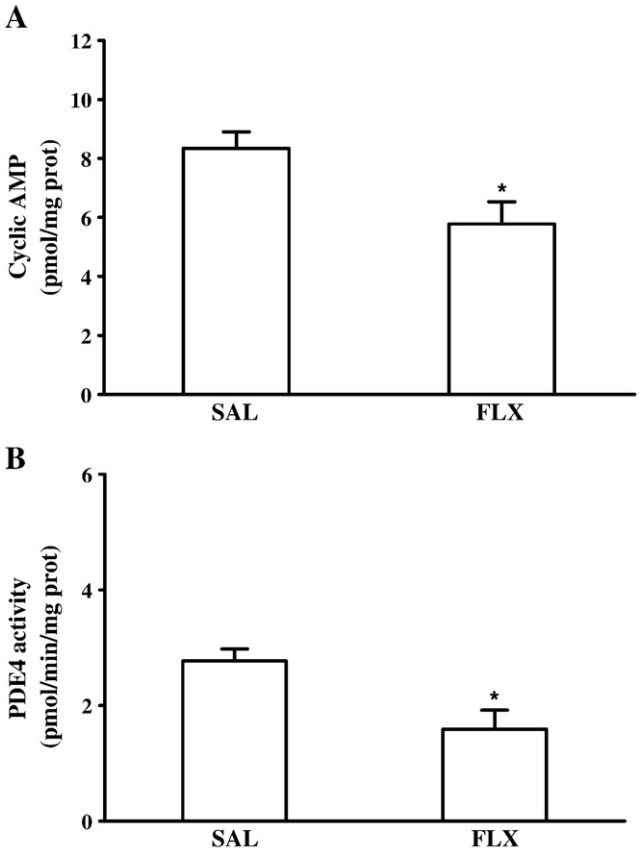

Fig. 3. Effect of fluoxetine or saline treatment on cAMP levels and PDE4 activity in the prefrontal cortex of HSB mice. HSB mice were treated chronically (21 days) with fluoxetine (FLX) or saline (SAL). Following sacrifice and tissue collection, prefrontal cortex samples were analysed for cAMP levels (A) and PDE4 activity (B). cAMP levels were determined from FLX- ($n=10$) or SAL treated ($n=5$) mice. Similarly, PDE4 activity was determined from FLX- ($n=5$) or SAL treated ($n=8$) animals. Significant differences versus control SAL are indicated by an asterisk (Students *T*-test; *$p<0.05$). Data shown represent the mean ± S.E.M.

striatum. In both instances, degree of stereotypy and cAMP levels demonstrated a small but positive association. In the prefrontal cortex, PDE4 activity was significantly reduced in both low and high stereotypic deer mice compared to their non-stereotypic controls. In the striatum, only high stereotypic mice showed a significant reduction in PDE4 activity. In both brain regions there was a significant inverse correlation between PDE4 activity and stereotypy, suggesting that in the prefrontal cortex especially, increased spontaneous stereotypy in deer mice is associated with reduced breakdown of cAMP by PDE4. Importantly, FLX significantly reduced stereotypy in high stereotypic animals together with a reduction in cortical cAMP levels and PDE4 activity, without noteworthy effects in the striatum.

Changes in brain 5-HT (Manji et al., 2001) and cAMP-PDE signalling represent an important component of a number of neuropsychiatric disorders (see Halene and Siegel, 2007 for review; Perez et al., 2000; Marazziti et al., 2001). 5-HT receptors link to cAMP through negative coupling to adenylate cyclase via 5-HT_{1A} receptors, or positive coupling via 5-HT_4, 5-HT_6 and 5-HT_7 receptors (Barnes and Sharp, 1999). An elevation in 5-HT following SRI administration will therefore lead to an associated decrease or increase in cAMP, depending on the relative density of the specific type of 5-HT receptor in the area under scrutiny (Barnes and Sharp, 1999). The PDE4 family, which solely hydrolyses cAMP, occupies a particularly prominent role in regulating cAMP signalling in the brain (Manji et al., 2001; Halene and Siegel, 2007). PDE activity is ubiquitously present in the cytosol of cells and neurons, as well as in a variety of membrane, nuclear and cytoskeletal locations (Houslay and Milligan, 1997; Soderling and Beavo, 2000) that contributes to the spatial and temporal characteristics of cAMP gradients within cells (Rich et al., 2001).

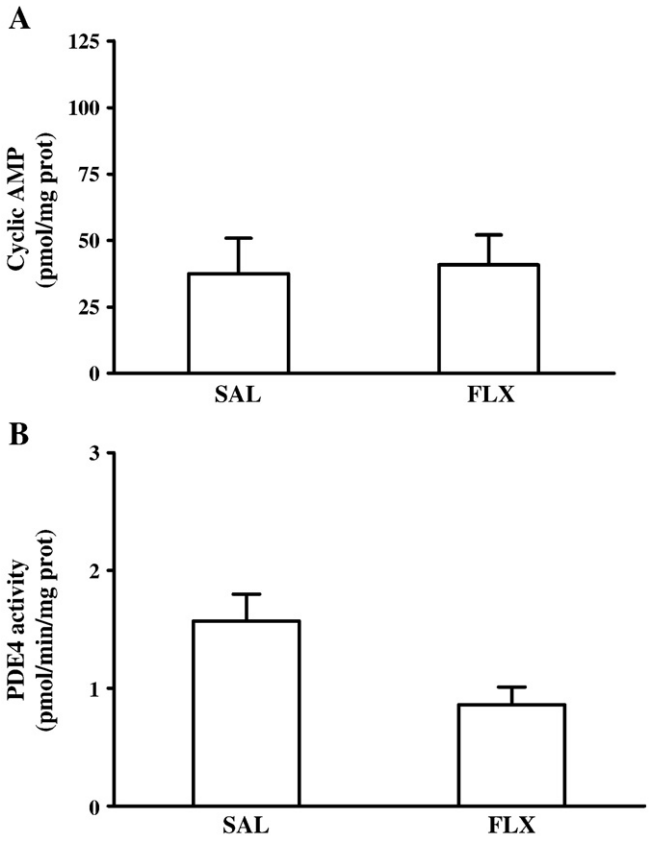

Fig. 4. Effect of fluoxetine or saline treatment on cAMP levels and PDE4 activity in the striatum of HSB mice. HSB mice were treated chronically (21 days) with fluoxetine (FLX) or saline (SAL). Following sacrifice and tissue collection, striatal samples were analysed for cAMP levels (A) and PDE4 activity (B). cAMP levels were determined from FLX- ($n=10$) or SAL ($n=13$) treated mice. Similarly, PDE4 activity was determined from FLX- ($n=5$) or SAL treated ($n=9$) animals. No significant differences versus SAL were noted (Students *T*-test). Data shown represent the mean ± S.E.M.

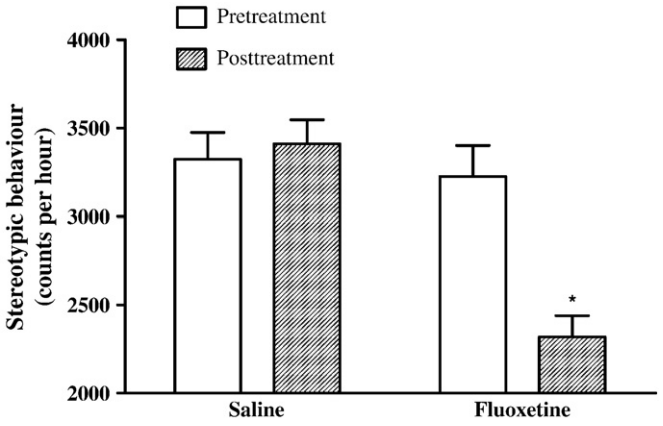

Fig. 5. Effect of fluoxetine or saline treatment on stereotypy in HSB mice on day 1 (baseline) and day 21 of treatment. HSB mice were treated chronically (21 days) with either fluoxetine (FLX) or saline (SAL). Stereotypy scores were determined as described in "Methods" for FLX-($n=10$) and SAL ($n=13$) treated animals. Significant differences between pre- and post FLX or SAL treatment are indicated by an *asterisk* (Students *T*-test; $*p=0.036$). Data shown represent the mean ± S.E.M.

Induced repetitive behaviours in response to amphetamine and its congeners, such as methylenedioxymethamphetamine or MDMA, includes head bobbing, patterned sniffing and gnawing (Kuczenski and Segal, 1989; Kuczenski et al., 1995), as well as head weaving and forepaw treading (Baumann et al., 2005). Studies with MDMA have noted that the latter stereotypies are highly correlated with increases in striatal and prefrontal cortex 5-HT (Baumann et al., 2008), while the selective 5-HT_{1A} agonist, 8-hydroxy DPAT, evokes a robust stereotypic response in rats (Hillegaart et al., 2000). Thus, increased 5-HT release in the prefrontal cortex and striatum, and subsequent activation of 5-HT_{1A} receptors in these regions, may underlie the *pharmacological* induction of stereotypy. In contrast, in a model of *spontaneous* stereotypy the non-specific 5-HT receptor agonist, m-chlorophenylpiperazine or m-CPP attenuates these behaviours (Korff et al., 2008). This raises the question whether the underlying mechanisms of induced and spontaneous stereotypy are fundamentally different, particularly with respect to the involvement of 5-HT. Earlier studies (Powell et al., 1999; Presti and Lewis, 2005; Korff et al., 2008) have confirmed the repetitive, unvarying and purposeless nature of deer mouse stereotypy (i.e. pattern running, somersaults, jumping) to be a separate behaviour from general increased locomotor activity. In the present study we have demonstrated that these same stereotypic behaviours are significantly inversely correlated with striatal and cortical PDE4 activity, with cAMP levels statistically higher in stereotypic animals compared with non-stereotypic animals. Thus, increased stereotypy in HSB mice was found to be linked to raised prefrontal cortical (but not striatal) cAMP and to reduced cortico-striatal PDE4 activity. Further, using chronic FLX administration, we have demonstrated that the ability of this SRI to reduce stereotypy involves the suppression of cAMP levels, but also suppression of PDE4 activity, in the prefrontal cortex of high stereotypic mice with little to no involvement of the striatum in this regard.

The response of cAMP-PDE4 signalling to SRI treatment observed in the present study is consistent with a previous literature noting that alteration of 5-HT levels leads to altered PDE4 expression and activity (Lourenco et al., 2006), and which may represent a compensatory response to activation of the cAMP system (Takahashi et al., 1999). Following chronic SRI administration, an initial inhibition of 5-HT reuptake results in an escalation of 5-HT levels in the mid-brain raphe nuclei and at distal projection synapses in

the cortex, striatum and hippocampus. Thereafter follows a gradual time-dependent desensitisation of pre-synaptic terminal 5-HT_{1A} receptors (Bergqvist et al., 1999; El Mansari and Blier, 2006) resulting in a sustained increase in 5-HT. Despite the evidence for a more dominant role for pre-synaptic 5-HT_{1A} receptors in SRI response (Rausch et al., 2006), the possible contributory role of *post-synaptic* antidepressant 5-HT_{1A} receptor regulation, and which may have opposing responses with respect to 5-HT release, also needs careful consideration (El Mansari and Blier, 2006). A number of earlier studies in rodents have noted that PDE4 expression is increased by chronic FLX administration (Takahashi et al., 1999; Zhao et al., 2003; D'Sa et al., 2005; Dlaboga et al., 2006). However, Miró et al. (2002) found that while mRNA levels for PDE4A were indeed increased in certain brain regions, mRNA for PDE4B, PDE4D and PDE1 was decreased in regions such as the frontal cortex, and Ye et al. (2000) found no change in the cortex following fluoxetine treatment. Thus, while unpredictable, it can be said that SRI-induced changes in the cAMP gradient will lead to regional-specific alterations in PDE4 expression and activity (Houslay et al., 1998; Conti and Jin, 1999; D'Sa and Duman, 2002). Indeed, in response to heightened cortical levels of cAMP observed in stereotypic deer mice, PDE4 may be recruited differently within select intracellular locations, e.g. the prefrontal cortex and striatum, and may respond differently to drug treatment.

Changes in cAMP were not observed in the striatum in drug-naive animals, while FLX treatment similarly did not illicit any significant effect on striatal cAMP levels in HSB animals, despite attenuating stereotypy. While the striatum is the main input structure of the basal ganglia and a key component of the motor system (Kelly, 1999), the prefrontal cortex is the dominant regulator of motor behaviour, exerting top-down control over behavioural responses originating in sub-cortical regions, such as the striatum (Dubois et al., 1994). In line with this, where cAMP levels were increased in the prefrontal cortex in drug naive high stereotypic animals, FLX significantly reduced these levels. That stereotypy was likewise reduced in these animals provides important evidence for a causal role for the prefrontal cortex in increased stereotypy and response to SRI treatment.

In the prefrontal cortex of HSB mice, FLX induced a significant reduction in PDE4 activity, with a trend to lower levels also noted in the striatum. This clearly is incongruent with its ability to reduce cAMP levels, especially in the prefrontal cortex. More in line with our data, however, Miró et al.(2002) have found that chronic FLX treatment decreases mRNA levels for a number of PDE4 isoforms. Moreover, with PDE4 activity found to be reduced in drug naive HSB mice, and FLX further reducing said activity, it is likely that the observed reductions in prefrontal cortical PDE4 activity following FLX treatment are a separate event that is not immediately involved in how the SRI may attenuate elevated cAMP levels and reduce stereotypy in these animals. It is possible that in high stereotypic deer mice, chronic FLX treatment may target elevated cortical cAMP signalling in a PDE4 independent manner, more likely involving serotonergic actions on Gi-dependent adenylate cyclase coupling. Nevertheless, it should be stated that a number of PDE's in the brain utilize cAMP as substrate (Beavo, 1995; Cote et al., 1999), including PDE1, PDE7, PDE8, and PDE10, such that actions on raised cAMP may involve various permutations of PDE enzyme activity. Protein expression and mRNA studies are needed to further elucidate the PDE4 isoforms involved in deer mouse stereotypy and in SRI response.

In conclusion, increased stereotypy in deer mice is characterized by raised cAMP levels in the prefrontal cortex (but not the striatum), and by reduced PDE4 enzyme activity in both prefrontal cortex and striatum. Chronic fluoxetine attenuated prefrontal cortex cAMP and PDE4 activity, and also reduced stereotypy, in high stereotypic animals. The attenuating action of fluoxetine on raised cAMP levels in these animals may be less dependent on its ability to alter PDE4 activity, perhaps involving 5-HT_{1A} receptor-Gi mediated attenuation of adenylate cyclase-cAMP signalling. Indeed, the possible role of altered adenylate cyclase activity in deer mouse stereotypy warrants further study.

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