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# Congenic $D_{1A}$ Dopamine Receptor Mutants: Ethologically Based Resolution of Behavioural Topography Indicates Genetic Background as a Determinant of Knockout Phenotype

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$D_{1A}$-null mice were backcrossed over 14 generations into a C57BL/6 background to result in essential elimination (to $<0.005\%$) of any contribution from the 129/Sv component of their initially mixed (129/Sv × C57BL/6) background. Their phenotype was assessed using an ethologically based approach that resolves each individual topography of behaviour in the natural repertoire. Habituation of sniffing, locomotion, rearing seated, and rearing to wall in wild types over several hours was profoundly retarded in congenic $D_{1A}$ mutants; conversely, rearing free and sifting were essentially abolished. Resultant increases in individual topographies of behaviour were substantially greater in congenic $D_{1A}$ mutants than in those on a mixed background. This phenotype was little altered by the selective $D_1$-like antagonist SCH 23390 and could not be blocked by the selective $D_2$-like antagonist YM 09151-2. The selective $D_1$-like agonist SK&F 83959 could not further elevate those behaviours already heightened in congenic $D_{1A}$ mutants, while the induction of stereotyped sniffing and plodding locomotion by the selective $D_2$-like agonist RU 24213 was disrupted. Genetic background appears to modulate critically the magnitude but not the general nature of the $D_{1A}$-null phenotype, which may involve compensatory processes independent of other $D_1$-like or $D_2$-like receptors.
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**Keywords:** dopamine $D_{1A}$ receptor; congenic $D_{1A}$ 'knockout'; genetic background; targeted gene deletion; behavioural phenotype; topographical assessment; ethogram

## INTRODUCTION

Although targeted gene deletion now allows the functional roles of dopamine (DA) receptor subtypes to be explored in the face of few agonists and antagonists able to discriminate materially between, for example, $D_{1A}/D_1$ *vs* $D_{1B}/D_5$ or $D_2$ *vs* $D_3$ *vs* $D_4$ receptors, this knockout approach presents its own practical and theoretical problems (Sibley, 1999; Waddington *et al*, 2001). One critical issue derives from the mixed (129/Sv × C57BL/6) genetic background on which essentially all DA receptor subtype (and most other) knockouts have been constructed and examined to date. Thus, it remains possible that phenotypic effects might reflect not only the entity deleted but also variations in that genetic background between individual animals; this issue may contribute to differences in findings between laboratories that have examined a knockout of a given receptor subtype, constructed using either the same or different gene targeting approaches (Gerlai, 1996; Crawley *et al*, 1997; Kelly *et al*, 1998; Phillips *et al*, 1999; Sanford *et al*, 2001; Waddington *et al*, 2001). Additionally, important if poorly understood differences between what are notionally 'similar' experimental paradigms applied in different laboratories (Crabbe *et al*, 1999) are recognised, and the examination of related aspects of phenotype using different experimental paradigms greatly exacerbates such problems (Waddington *et al*, 2001).

In relation to $D_{1A}$ mutants, there are now several phenotypic studies at the level of behaviour which, on a mixed genetic background and utilising a diversity of behavioural approaches, have indicated a number of discrepant findings (Drago *et al*, 1994; Xu *et al*, 1994a,b; Clifford *et al*, 1998; Cromwell *et al*, 1998; Smith *et al*, 1998; Waddington *et al*, 2001). For example, one study has reported some increase in otherwise undifferentiated activity in terms of photobeam interruptions (Xu *et al*,

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1994a,b), while other studies have reported no change (Drago *et al*, 1994) or even some reduction (Smith *et al*, 1998) in variously defined 'locomotor activity' on such a mixed background. One approach to the issue of genetic background involves repeated backcrossing onto a single strain, usually C57BL/6, but the time and effort involved in attaining congenicity has so far restricted its application in relation to DA receptor subtypes; specifically, there is, to date, little in the way of systematically collected data other than in relation to the $D_2$ receptor using incipient congenic knockouts (five backcrosses; Kelly *et al*, 1998). The assessment of behavioural phenotype in $D_{1A}$ knockouts following 14 backcrosses into a C57BL/6 background to attain essential elimination (to $<0.005\%$) of any contribution from the 129/Sv component of their initially mixed background, other than the transgene itself, is described here. Furthermore, we have applied an ethologically based approach to resolve all topographies of behaviour within the mouse repertoire, to allow comparison with the phenotype of the identical $D_{1A}$ knockout on its original mixed (129/Sv $\times$ C57BL/6) background as evaluated in this laboratory using the same approach (Clifford *et al*, 1998; Waddington *et al*, 2001).

## METHODS

### Targeted Gene Deletion

The original hybrid strain (129/Sv $\times$ C57BL/6) containing the mutated $D_{1A}$ receptor allele was generated as reported previously (Drago *et al*, 1994). In outline, the targeted gene deletion was constructed in 129/Sv embryonic stem cells and male chimeras mated with C57BL/6 females to produce heterozygous mutants [$D_{1A}^{+/-}$]; homozygous mutants [$D_{1A}^{-/-}$] and wildtypes [$D_{1A}^{+/+}$] were identified among the progeny of heterozygous intermatings using Southern blotting of isolated tail DNA. To establish an essentially congenic line of $D_{1A}$ knockouts, heterozygous mutants of this hybrid (129/Sv $\times$ C57BL/6) strain were backcrossed to wildtype C57BL/6 for seven generations. Heterozygous mutants of this seventh generation were then shipped to Dublin; here, this procedure was continued for an additional seven generations, giving a total of 14 backcrosses to wildtype C57BL/6. Analysis of isolated tail DNA by PCR was used to identify congenic, homozygous mutants and wildtypes among the progeny of heterozygous intermatings.

Mice were housed in groups of five with food and water available *ad libitum*, and were maintained at $21 \pm 1^{\circ}$C on a 12 h/12 h (0900 on/2100 off) light/dark schedule. As a result of a well-documented, early failure to thrive over the weaning period among $D_{1A}$ knockouts on a mixed genetic background, resulting in a modest decrease in body weight (Drago *et al*, 1994; Xu *et al*, 1994a,b), standard dry mouse chow was supplemented routinely with moist diet on the floor of the cage. Young male or female mice from litters of the same generational age were used. These studies were approved by the Research Committee of the Royal College of Surgeons in Ireland and were conducted under licence from the Department of Health & Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

### Topographical Assessment of Behaviour

For evaluation of *ethograms* in terms of spontaneous behavioural topography under the condition of active exploration (unhabituated condition), mice were removed from their home cage and placed individually in clear glass observation cages ($36 \times 20 \times 20$ cm). Assessments were carried out using a rapid time-sampling behavioural checklist technique, in a manner similar to that described previously (Clifford *et al*, 2000, 2001). For this procedure, each of 10 randomly allocated mice was observed for 5-s periods at 1-min intervals over 15 consecutive minutes, using an extended, ethologically based behavioural checklist to allow the presence or absence of the following individual behaviours (occurring alone or in any combination) to be determined in each 5-s period: sniffing (flaring of nostrils with movement of vibrissae); locomotion (coordinated movement of all four limbs producing a change in location); total rearing (of any form); rearing seated (front paws reaching upwards with hindlimbs on the floor in a sitting position); rearing free (front paws reaching upwards away from any cage wall while standing on hindlimbs); rearing to wall (front paws reaching upwards onto or towards a cage wall while standing on hindlimbs); sifting (characteristic sifting movements of the forepaws through cage bedding material on cage floor); grooming (of any form); intense grooming (characteristic pattern of grooming of the snout and then face with the forepaws, followed by vigorous grooming of the hind flank or anogenital region with the snout); chewing (chewing movements directed onto physical material, that is, cage bedding and/or faecal pellets, without consumption); stillness (asleep or motionless, with no behaviour evident). Levels of vacuous chewing (chewing movements not directed onto any physical material), eating (chewing with consumption), and climbing (jumping onto cage top with climbing along grill in inverted or hanging position) were too low for meaningful assessment. This cycle of assessment by the behavioural checklist over a 15-min period (0–15 min) was repeated twice (20–35 and 40–55 min). For continuing evaluation of subsequent habituation of spontaneous exploratory behavioural topography, $8 \times 10$-min cycles of otherwise identical assessments were repeated at 80–90, 120–130, 160–170, 200–210, 240–250, 280–290, 340–350, and 360–370 min. Under these conditions, mice were used on a single occasion, comprising a complete 0–370 min observation schedule, with all assessments made by an observer who was unaware of the genotype of each animal.

Evaluation of *ethograms* following pretreatment with agonists or antagonists utilised procedures similar to those described earlier. However, in these experiments, animals were habituated to identical observation cages for a period of 3 h; this was to reduce initially high levels of activities and to reveal late phenotypic effects (see Results), in order to optimise detecting any stimulatory effects of agonists and attenuating effects of antagonists. Immediately following pretreatment with drug or vehicle, each of the 10 randomly allocated mice was observed individually with an otherwise identical behavioural checklist supplemented to include ponderous locomotion, a 'plodding' variant induced in mice by $D_2$-like agonists that differs from the more normal, fluid ambulation induced in rats (see Clifford *et al*, 1999, 2000,

2001); after a 15-min assessment using the checklist, each animal was evaluated over a 30-s period using a conventional 0 to 6-point stereotypy scale: 0 = asleep or inactive; 1 = episodes of normal activity; 2 = discontinuous activity with bursts of prominent sniffing or rearing; 3 = continuous stereotyped activity such as sniffing or rearing along a fixed path; 4 = stereotyped sniffing or rearing fixated in one location; 5 = stereotyped behaviour with bursts of licking or gnawing; 6 = continuous licking or gnawing. This cycle of assessment by behavioural checklist followed by stereotypy scale was repeated on two further occasions over a total period of 1 h. Under these conditions, mice were given agonists on two occasions only, separated by a drug-free interval of at least 1 week. For antagonists, conservation of mutants in limited supply necessitated their use in two studies: mice were given one antagonist on two occasions separated by a drug-free interval of at least 1 week and, following a drug-free washout period of 1 month, were 'quasirandomly' assigned to treatment with the second antagonist, as detailed below. Animals receiving SK&F 83959 had been assessed 1 week previously for their *ethogram* in terms of spontaneous behavioural topography, while those receiving other treatments had not; however, all animals received the same period of habituation immediately prior to drug treatment. On each occasion, mice were allocated randomly to one of the various treatment groups, with all assessments made by an observer who was unaware of the genotype and treatment for each animal.

### Drugs

The following drugs were used: SCH 23390 ([*R*]-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine; Research Biochemicals International, USA); YM 09151-2 (*cis*-*N*-[1-benzyl-2-methyl-pyrrolidin-3-yl]-5-chloro-2-methoxy-4-methylaminobenzamide; Yamanouchi, Japan); SK&F 83959 (3-methyl-6-chloro-7,8-dihydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepine; Research Biochemicals International/NIMH Chemical Synthesis Program, USA); RU 24213 (*N*-*n*-propyl-*N*-phenylethyl-*p*-3-hydroxyphenylethylamine; Hoechst-Marion-Roussel, France). SCH 23390, SK&F 83959, and RU 24213 were dissolved in distilled water; YM 09151-2 was dissolved in 0.1 N HCl and made up to volume with distilled water. All drugs and their respective vehicles were injected subcutaneously into the flank in a volume of 2 ml/kg.

### Data Analysis

As described previously (Clifford *et al*, 1999, 2000, 2001), for specification of *ethograms* for spontaneous behavioural topography over a phase of initial exploratory activity, the total 'counts' for each individual behaviour were determined as the number of 5-s observation windows in which a given behaviour was evident, summed over the initial $3 \times 15$-min (0–15, 20–35, and 40–55 min) cycle periods, and expressed as means $\pm$ SEM. For determination of the habituation profiles of these *ethograms*, total 'counts' for each individual behaviour were summed as above over each of the following periods: 0–10, 20–30, 40–50, 80–90, 120–130, 160–170, 200–210, 240–250, 280–290, 340–350, and 360–370 min; these were expressed also as means $\pm$ SEM. For specification of *ethograms* for drug-induced behavioural topography, the total 'counts' for each individual behaviour were determined as the number of 5-s observation windows in which a given behaviour was evident, summed over the initial $3 \times 15$-min (0–15, 20–35, 40–55 min) cycle periods; data were collapsed across the two test sessions, and expressed as means $\pm$ SEM. Stereotypy scores were averaged over the 1-h period and expressed similarly. 'Counts' for individual behaviours were analysed using a separate ANOVA for each behaviour, followed by Student's *t*-tests to identify individual group differences contributing to significant overall effects on ANOVA; in instances where data distribution deviated from normality, the Kruskal–Wallis nonparametric ANOVA was used, followed by Mann–Whitney U-tests. Stereotypy scores were analysed using the Kruskal–Wallis nonparametric ANOVA, followed by Mann–Whitney U-tests. In the absence of appropriate nonparametric techniques, interaction effects were analysed using ANOVA following square-root transformation (Clifford *et al*, 1999, 2000, 2001).

## RESULTS

### General Parameters: Spontaneous Behaviour

On examining 39 (20 females, 19 males) congenic $D_{1A}$-null mice, the mean body weight ($17 \pm 1$ g, mean age $153 \pm 4$ days) was reduced (−35%, $p < 0.001$) relative to 39 (20 females, 19 males) wildtype controls ($26 \pm 1$ g, mean age $153 \pm 4$ days). On qualitative inspection of posture, reactivity to handling and general activity, no gross motor phenotype was apparent.

Ethogram *of spontaneous behaviour over exploratory phase* On comparison with wildtypes, congenic $D_{1A}$-null mice were characterised over the initial 1-h exploratory phase by increased locomotion (+79%, $p < 0.001$] with decreased sifting (−93%, $p < 0.001$) and total grooming (−32%, $p < 0.01$] (Figure 1); although total counts for rearing were unaltered, rearing seated was increased (+49%, $p < 0.05$) while rearing free was reduced (−95%, $p < 0.001$). Raised scores on the stereotypy scale were confined to the lower end of the 0–6 range (mean scores: wildtypes $2.0 \pm 0.0$, $D_{1A}$-null $2.4 \pm 0.1$, $p < 0.001$); this indicated a heightening of normal behaviours rather than a transition to stereotyped behaviour. No other effects of genotype were evident. There were overall effects of gender for sniffing (females > males, $p < 0.001$), sifting (males > females, $p < 0.01$), rearing seated (females > males, $p < 0.01$), and total grooming (males > females, $p < 0.01$), but no gender × genotype interactions other than increased rearing seated among mutants being modestly greater ($p < 0.05$) in females than in males.

Ethogram *of spontaneous behaviour over habituation phase* Each individual topography of behaviour, with the exception of grooming, habituated readily over the total period of 370 min, there being significant effects of time bins ($p < 0.001$) for sniffing, locomotion, sifting, total rearing, rearing free, rearing to wall, and rearing seated (Figure 2a,b); total grooming also varied with time bins, but in a manner distinct from habituation. Congenic $D_{1A}$-null

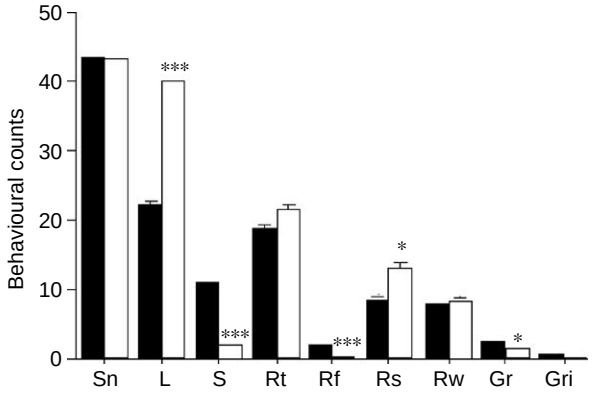


**Figure 1** Topography of spontaneous behaviour over an initial 60-min exploratory period. Data are mean behavioural counts ± SEM for sniffing (Sn), locomotion (L), sifting (S), total rearing (Rt), rearing free (Rf), rearing seated (Rs), rearing to wall (Rw), total grooming (Gr), and intense grooming (Gri) for wild type ($n = 39$ (19 males, 20 females), filled columns) and congenic $D_{1A}$-null ($n = 39$ (19 males, 20 females), open columns) mice. ***$p < 0.001$, *$p < 0.05$ *vs* wildtype.

mice failed to habituate to the same extent as their wildtype counterparts, resulting in behaviours that were manifested at heightened levels in the absence of stereotypy; there were overall effects of genotype ($p < 0.001$) and time × genotype interactions ($p < 0.001$) for each of sniffing, locomotion, rearing total, rearing to wall, and rearing seated. Conversely, sifting and rearing free each declined over time bins in wildtypes but were essentially abolished in $D_{1A}$-null mice (effects of genotype and time × genotype interactions, $p < 0.001$), while grooming was reduced in $D_{1A}$-null mice over the habituation phase (effect of genotype, $p < 0.001$). Although there were effects of gender over habituation for sniffing, locomotion, sifting, total rearing, rearing seated, and total grooming, as noted above for the initial exploratory phase, time × genotype × gender interactions were evident only for sniffing and locomotion; for these behaviours, delayed habituation in $D_{1A}$-null mice was modestly greater ($p < 0.05$) in females than in males.

*Comparison of* Ethograms *for spontaneous behaviour over habituation phase between congenic and hybrid $D_{1A}$ mutants* The above habituation profile in congenic $D_{1A}$-null mice was compared with the habituation profile in hybrid (mixed background 129/Sv × C57BL/6) $D_{1A}$-null mice from which this congenic line was generated (see Methods), as obtained in this laboratory under similar conditions using the same assessment techniques (Clifford *et al*, 1998; Waddington *et al*, 2001; Figure 2a,b). For sniffing, similar initial levels habituated more slowly in congenic wildtypes than in their hybrid counterparts; although hybrid $D_{1A}$ mutants habituated somewhat less readily, congenic $D_{1A}$ mutants were characterised by the essential absence of habituation to result in markedly higher levels over later time bins (time × genotype × background interaction, $p < 0.001$). For locomotion, wildtypes from each background evidenced similar initial levels and rates of habituation; while hybrid $D_{1A}$ mutants habituated similarly as their wildtype counterparts from their elevated baseline, congenic $D_{1A}$ mutants were characterised by the essential absence of habituation to result in markedly higher levels of locomotion over later time bins (time × genotype × background interaction, $p < 0.001$).

For total rearing, wildtypes on each background evidenced generally similar initial levels and rates of habituation; while hybrid $D_{1A}$ mutants habituated slightly less readily than their wildtype counterparts, congenic $D_{1A}$ mutants were characterised by the essential absence of habituation to result in markedly higher levels over later time bins (time × genotype × background interaction, $p < 0.001$). For rearing free, lower initial levels in congenic wildtypes habituated at a rate similar to that of their hybrid counterparts; $D_{1A}$ mutants on each background evidenced lower initial levels but similar habituation profiles relative to their wildtype counterparts (no time × genotype × background interaction). For rearing seated, higher initial levels in congenic wildtypes habituated at a rate similar to that of their hybrid counterparts; in hybrid $D_{1A}$ mutants, there was little habituation of this low baseline, while in congenic $D_{1A}$ mutants levels of rearing seated not only failed to habituate but increased further to result in markedly higher levels over later time bins (time × genotype × background interaction, $p < 0.001$). For rearing to wall, wildtypes from each background evidenced similar initial levels and rates of habituation; congenic $D_{1A}$ mutants habituated more slowly than their hybrid counterparts to result in higher levels over later time bins (time × genotype × background interaction, $p < 0.001$).

For sifting, wildtypes from each background evidenced generally similar initial levels and rates of habituation; congenic $D_{1A}$ mutants evidenced substantially lower levels than their hybrid counterparts (time × genotype × back- × background interaction, $p < 0.01$). Total grooming was increased in hybrid but reduced in congenic $D_{1A}$ mutants across time bins; a similar profile was evident for intense grooming, primarily over early time bins (time × genotype × background interaction, $p < 0.05$).

## General Parameters: Effects of SCH 23390 and YM 09151-2

On examining 20 female congenic $D_{1A}$-null mice, the mean body weight (14 ± 1 g, mean age 193 ± 17 days) was reduced (−36%, $p < 0.001$) relative to 20 female wildtype controls (22 ± 1 g, mean age 202 ± 21 days).

Ethogram *following pretreatment with the $D_1$-like antagonist SCH 23390* Over a 1-h period, pretreatment of wildtypes with 0.005–0.625 mg/kg SCH 23390 readily and dose-dependently reduced sniffing, locomotion, total rearing, rearing seated, rearing to wall, and total grooming (Figure 3). For those behaviours found to be heightened over this period in congenic $D_{1A}$-null mice in the absence of any treatment, namely locomotion and rearing seated, similar effects were noted here following vehicle pretreatment; these heightened behaviours were unaltered by SCH 23390 (effects of genotype and genotype × treatment interactions, $p < 0.005$). Sniffing, total rearing, and rearing to wall were also essentially unaltered by SCH 23390 in $D_{1A}$ mutants, such that at higher doses of SCH 23390 they occurred to excess relative to wildtypes (effects of genotype and genotype × treatment interactions: sniffing and total

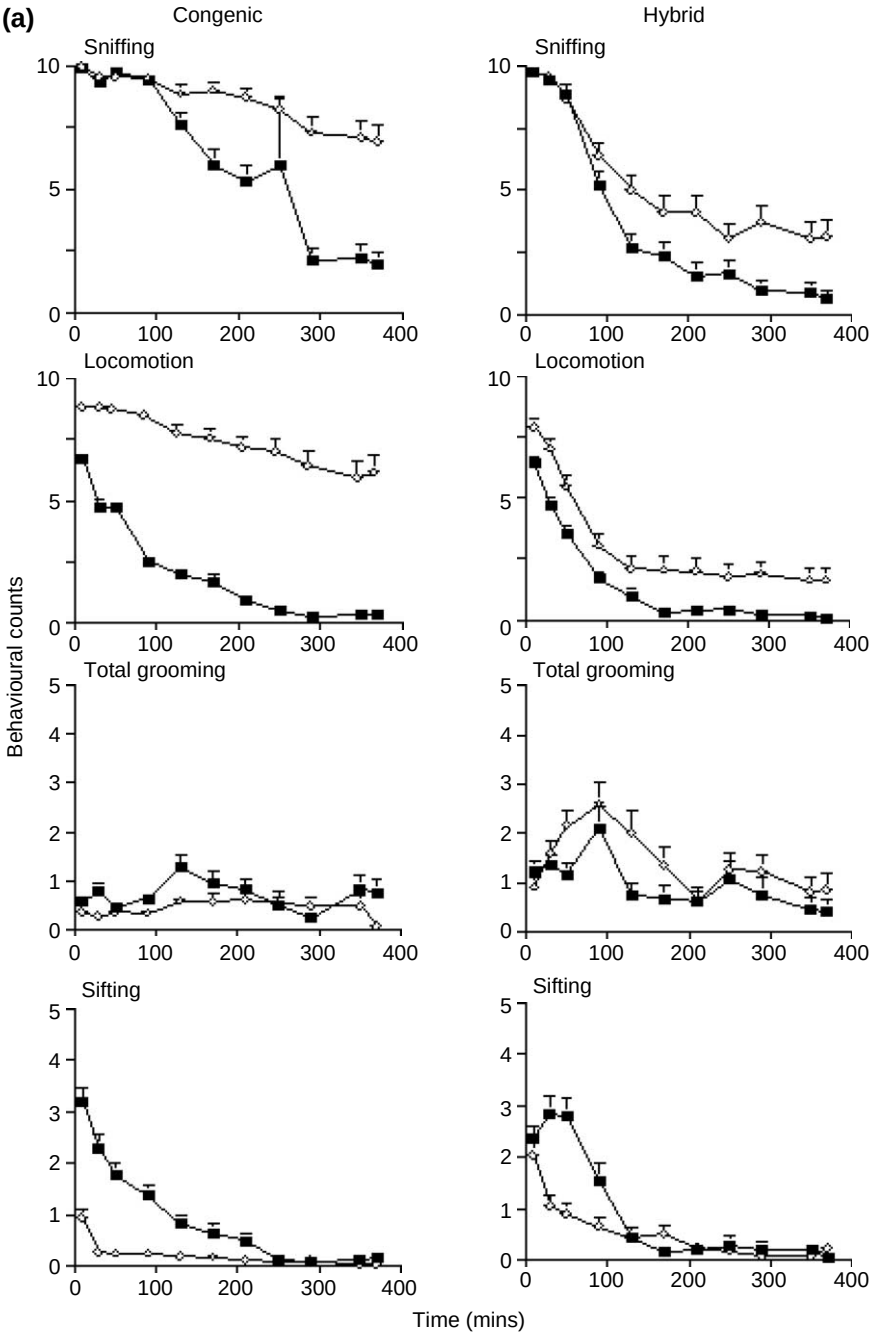


**Figure 2** Topographical assessment of spontaneous behaviour over a 370-min phase of habituation. (a) Data are mean behavioural counts ± SEM per 10-min time bin for sniffing, locomotion, total grooming, and sifting, for (left column) wildtype ($n=39$ (19 males, 20 females), filled symbols) and congenic $D_{1A}$-null ($n=39$ (19 males, 20 females), open symbols) mice. For comparison (right column) are juxtaposed data for wildtype ($n=38$ (17 males, 21 females), filled symbols) and $D_{1A}$-null ($n=39$ (20 males, 19 females), open symbols) mice on original mixed genetic background (Clifford *et al* (1998), reproduced with permission from Elsevier Science). (b) Data are mean behavioural counts ± SEM per 10-min time bin for total rearing, rearing free, rearing seated, and rearing to wall, for (left column) wildtype ($n=39$ (19 males, 20 females), filled symbols) and congenic $D_{1A}$-null ($n=39$ (19 males, 20 females), open symbols) mice. For comparison (right column) are juxtaposed data for wildtype ($n=38$ (17 males, 21 females), filled symbols) and $D_{1A}$-null ($n=39$ (20 males, 19 females), open symbols) mice on original mixed genetic background (Clifford *et al* (1998), reproduced with permission from Elsevier Science).

rearing, $p<0.001$; rearing to wall, $p<0.05$). For those behaviours found above to be reduced over this period in congenic $D_{1A}$-null mice in the absence of any treatment, namely sifting and total grooming, similar effects were noted here following vehicle pretreatment; sifting was essentially abolished in $D_{1A}$ mutants pretreated with vehicle but re-emerged following pretreatment with SCH 23390 to exceed the levels in wildtypes (genotype × treatment interaction, $p<0.005$); total grooming was lower in $D_{1A}$ mutants pretreated with vehicle, with this lower level essentially unaltered by SCH 23390 such that at higher doses grooming was in

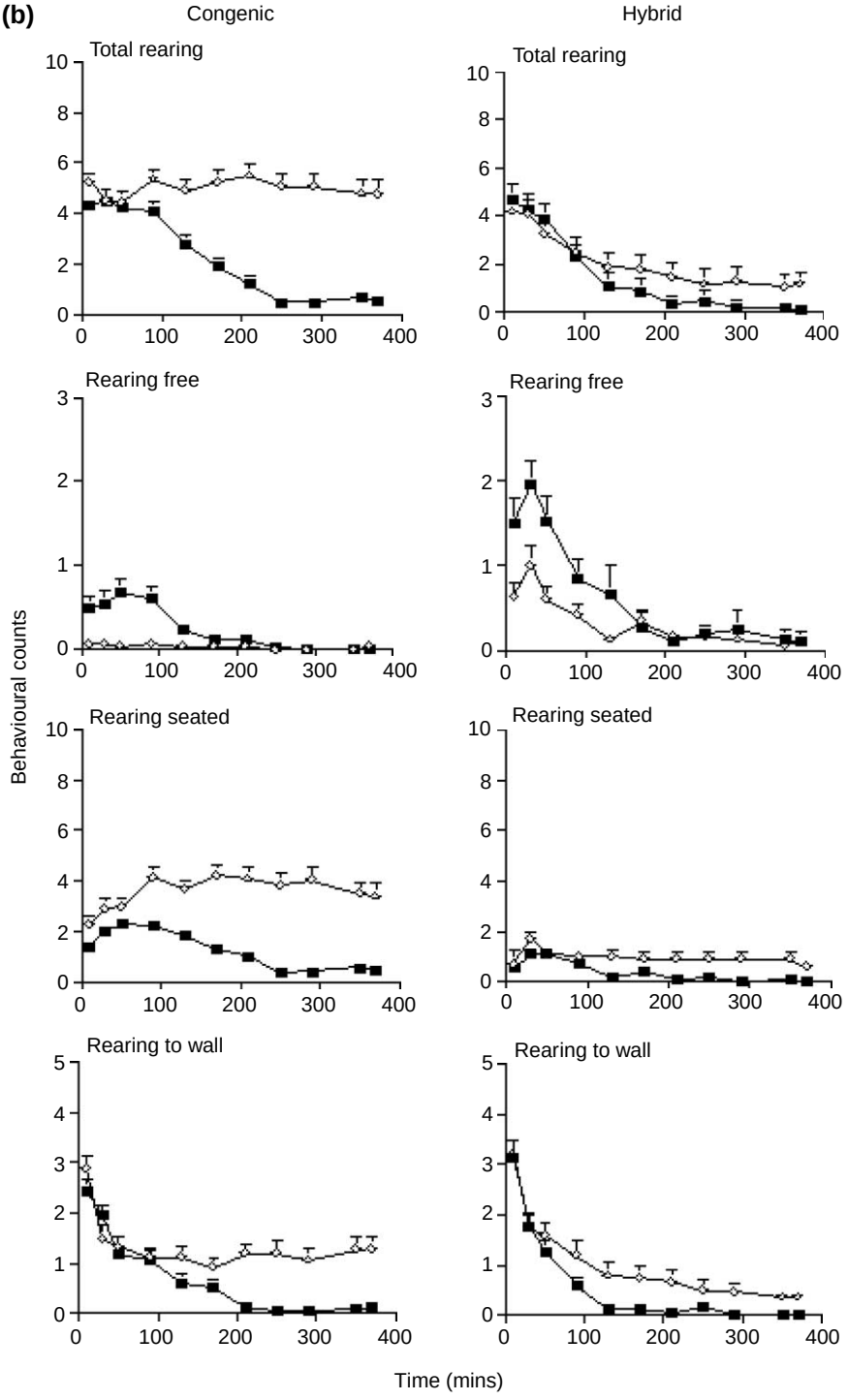


**Figure 2** (continued).

excess relative to wildtypes (effect of genotype and genotype × treatment interaction, $p<0.001$).

Ethogram *following pretreatment with the $D_2$-like antagonist YM 09151-2* Following the completion of studies with SCH 23390, the above animals were left drug-free for a washout period of 1 month before conducting similar studies with YM 09151-2. Over a 1-h period, pretreatment of wildtypes with 0.005–0.625 mg/kg YM 09151-2 dose-dependently reduced sniffing, locomotion, total rearing, rearing seated, and total grooming (Figure 4); baseline levels, particularly of sifting, were lower than in the preceding study with SCH 23390, suggesting further habituation of this exploratory behaviour consequent to prior exposure to the test environment, with lower doses of YM 09151-2 increasing and higher doses decreasing sifting. For those

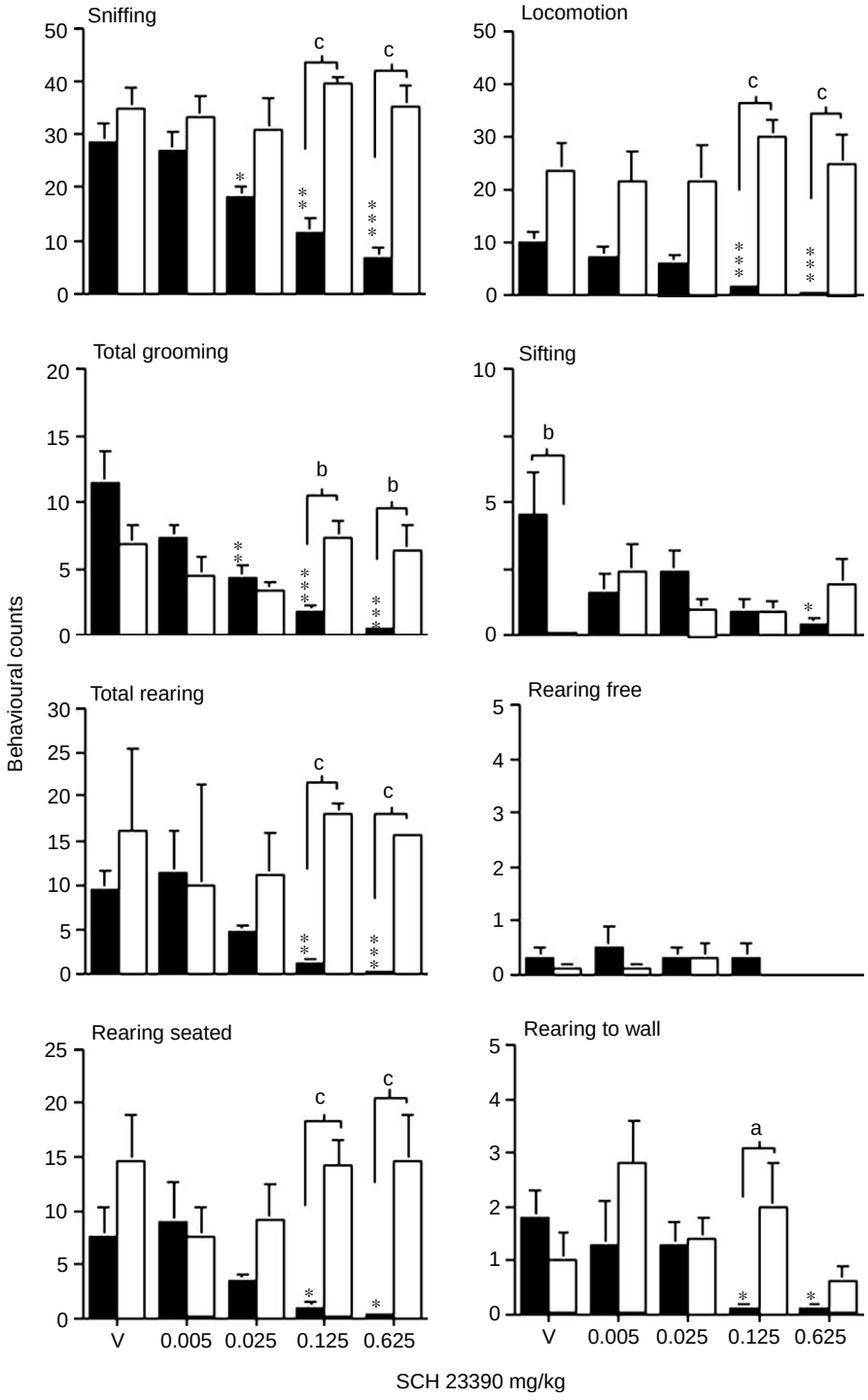


**Figure 3** Topographical effects of pretreatment with 0.005–0.625 mg/kg SCH 23390 or vehicle (V) following 3 h of habituation. Data are mean behavioural counts ± SEM over a 60-min period, for sniffing, locomotion, total grooming, sifting, total rearing, rearing free, rearing seated, and rearing to wall, for wildtype ($n = 20$ females, filled columns) and congenic $D_{1A}$-null ($n = 20$ females, open columns) mice. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs* vehicle-treated control of the same genotype; [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ between genotypes receiving the same dose.

behaviours found to be heightened over this period in congenic $D_{1A}$-null mice in the absence of any treatment, namely locomotion and rearing seated, similar effects were noted here following vehicle pretreatment, particularly for locomotion; heightened locomotion in $D_{1A}$ mutants was attenuated by YM 09151, as was rearing seated, but overall levels remained elevated relative to wildtypes (locomotion: effects of genotype and of treatment, $p < 0.001$; rearing seated: effect of treatment, $p < 0.001$). Sniffing and total rearing were attenuated by YM 09151-2 in $D_{1A}$ mutants (effects of treatment, $p < 0.001$), but overall levels of sniffing, and also of rearing to wall, remained elevated relative to wildtypes (effects of genotype, $p < 0.01$). For those behaviours found above to be reduced over this

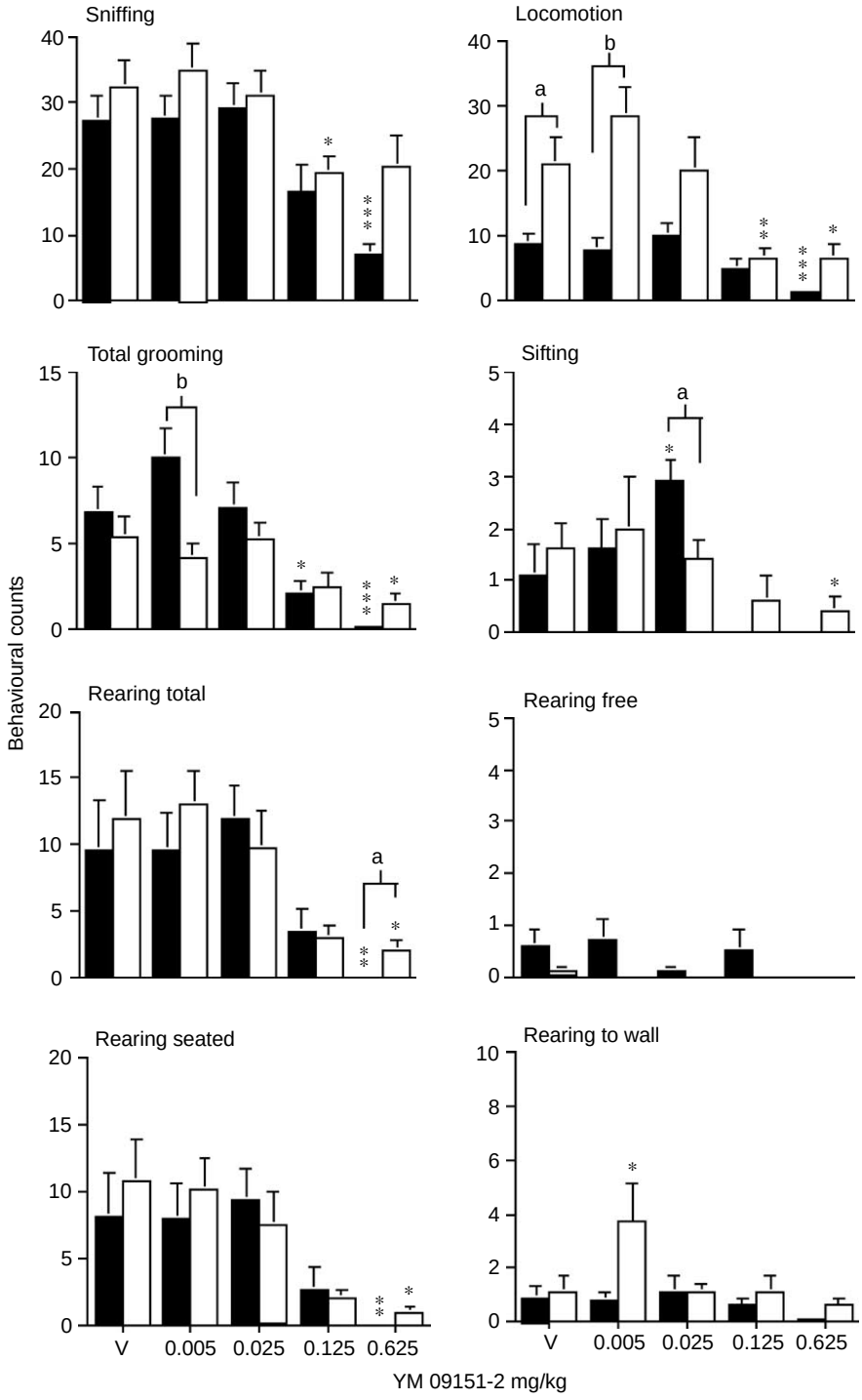


**Figure 4** Topographical effects of pretreatment with 0.005–0.625 mg/kg YM 09151-2 or vehicle (V) following 3 h of habituation. Data are mean behavioural counts ± SEM over a 60-min period, for sniffing, locomotion, total grooming, sifting, total rearing, rearing free, rearing seated, and rearing to wall, for wildtype ($n = 20$ females, filled columns) and congenic $D_{1A}$-null ($n = 20$ females, open columns) mice. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs* vehicle-treated control of the same genotype; [a]$p < 0.05$, [b]$p < 0.01$ between genotypes receiving the same dose.

period in $D_{1A}$ mutants in the absence of any treatment, namely sifting and total grooming, no such effects were noted here following vehicle pretreatment, suggesting some consequence of prior exposure to the test environment and/or stress of vehicle challenge; sifting was attenuated by YM 09151-2 in $D_{1A}$ mutants in a manner similar to wildtypes (effect of treatment, $p < 0.001$; no effect of genotype or genotype × treatment interaction), while grooming was attenuated more readily in wildtypes than in $D_{1A}$ mutants (effect of treatment, $p < 0.001$; genotype × treatment interaction, $p < 0.001$).

### General Parameters: Responsivity to SK&F 83959

On examining 20 female congenic $D_{1A}$-null mice, the mean body weight (16 ± 1 g, mean age 177 ± 9 days) was reduced

(−27%, $p<0.001$) relative to 20 female wildtype controls (22 ± 1 g, mean age 183 ± 9 days).

Ethogram *of responsivity to the selective $D_1$-like agonist SK&F 83959* Over a 1-h period, congenic $D_{1A}$-null mice pretreated with vehicle showed heightened levels of sniffing, locomotion, total rearing, and rearing seated, with reduction in sifting, relative to their wildtype counterparts (Figure 5). Challenge with 0.016–2.0 mg/kg SK&F 83959 in wildtypes readily induced sniffing, locomotion, total grooming and particularly intense grooming, total rearing, rearing free, and rearing seated, which attained only threshold levels of stereotypy. Conversely, in vehicle-treated $D_{1A}$ mutants elevated baseline levels of sniffing and locomotion could not be elevated further by any dose, such that SK&F 83959 increased these behaviours in wildtypes until they reached, but did not exceed, $D_{1A}$ mutant levels (effects of treatment, $p<0.005$; effects of genotype, $p<0.02$; genotype × treatment interactions, $p<0.001$); there were similar profiles for total rearing and rearing seated (genotype × treatment interactions, $p<0.05$). Induction of total grooming and particularly of intense grooming was reduced in $D_{1A}$ mutants (effects of treatment, $p<0.02$; effects of genotype, $p<0.01$). Induction of rearing free was essentially absent in $D_{1A}$ mutants (effect of genotype, $p<0.001$; genotype × treatment interaction, $p<0.02$), with induction of rearing to wall somewhat attenuated (genotype × treatment interaction, $p<0.02$). SK&F 83959 failed to induce sifting, which was essentially absent in $D_{1A}$ mutants (effect of genotype, $p<0.001$).

### General Parameters: Responsivity to RU 24213

On examining 19 female congenic $D_{1A}$-null mice, the mean body weight (16 ± 1 g, mean age 152 ± 6 days) was reduced (−27%, $p<0.001$) relative to 20 female wildtype controls (22 ± 1 g, mean age 161 ± 7 days).

Ethogram *of responsivity to the selective $D_2$-like agonist RU 24213* Over a 1-h period, congenic $D_{1A}$-null mice pretreated with vehicle showed heightened levels of sniffing, locomotion, total rearing, rearing seated, and rearing to wall, with reduction in total grooming, relative to their wildtype counterparts (Figure 6). Following challenge with 0.1–12.5 mg/kg RU 24213 in wildtypes, lower doses reduced sniffing, locomotion, rearing, and grooming, while higher doses readily induced stereotyped sniffing and ponderous locomotion with further decreases in other elements of behaviour.

Elevated baseline levels of sniffing in vehicle-treated congenic $D_{1A}$ mutants could not be increased further by any dose, such that RU 24213 increased these behaviours in wildtypes until they reached, but did not exceed, the level in $D_{1A}$ mutants (effect of treatment, $p<0.001$; effect of genotype, $p<0.001$; genotype × treatment interaction, $p<0.005$). For each of locomotion, total rearing, rearing seated, and rearing to wall, elevated baseline levels in vehicle-treated $D_{1A}$ mutants were unaltered by lower doses but reduced by higher doses of RU 24213 (effects of treatment, $p<0.02$; effects of genotype, $p<0.001$; genotype × treatment interactions, $p<0.05$); this reduction in overall locomotion in $D_{1A}$ mutants by RU 24213 was accompanied by induction of plodding locomotion to a level which at low-mid doses exceeded that induced in wildtypes but at the highest dose declined to a level below that induced in wildtypes (effect of treatment, $p<0.001$; genotype × treatment interaction, $p<0.005$). A lower baseline level of grooming in vehicle-treated $D_{1A}$ mutants was reduced further by RU 24213 in a manner similar to wildtypes (effect of treatment, $p<0.001$). While sifting was reduced similarly for both genotypes (effect of treatment, $p<0.001$), RU 24213 failed to induce chewing in wildtypes but did so readily in $D_{1A}$ mutants (effect of treatment, $p<0.001$; effect of genotype, $p<0.001$; genotype × treatment interaction, $p<0.001$). Elevated baseline stereotypy scores in vehicle-treated congenic $D_{1A}$ mutants were increased further by RU 24213, to remain above the levels induced in wildtypes (effect of genotype, $p<0.001$; effect of treatment, $p<0.001$).

## DISCUSSION

At the level of spontaneous behaviour, the *ethogram* of congenic $D_{1A}$-null mice over an initial exploratory period was characterised primarily by a marked increase in ethologically defined locomotion. This increase (+79%) was more than double that (+35%) in identical $D_{1A}$ mutants on their original mixed genetic background that we have reported previously using the same assessment technique (Clifford *et al*, 1998; Waddington *et al*, 2001), which itself appeared to complement a previous report of some increase in an otherwise undifferentiated activity in terms of photobeam interuptions (Xu *et al*, 1994a,b); however, other studies have reported no change (Drago *et al*, 1994) or even some reduction (Smith *et al*, 1998) in variously defined 'locomotor activities' on such a mixed background.

A shift in topography of rearing from rearing free to rearing seated was more complex than a previously noted reduced level of overall rearing events (Drago *et al*, 1994) or the selective reduction in rearing free that we have reported previously (Clifford *et al*, 1998; Waddington *et al*, 2001), each on a mixed genetic background; this indicates how compositing distinct topographies of rearing into an overall category can obscure subtle aspects of phenotype. Reduction in sifting was considerably more marked in congenic $D_{1A}$ mutants (−93%) than in those on a mixed background (−52%). If increased locomotion were accompanied by increases in sifting and rearing free, this would suggest heightened exploratory activities; however, increased locomotion was accompanied by decreases in sifting and rearing free, with a topographical shift to rearing seated, suggesting increased locomotor drive. The present decrease in grooming in congenic $D_{1A}$ mutants is contrary to our previous report of increased grooming and intense grooming on a mixed genetic background (Clifford *et al*, 1998); this difference may reflect, at least in part, the considerably greater increase in locomotion here which is physiologically antagonistic to, and hence disruptive of, more subtle grooming syntax within a response incompatibility model of behavioural topography (Waddington *et al*, 2001).

However, on extending these assessments beyond the period of initial exploration to include several hours of habituation thereafter, a much more profound phenotype

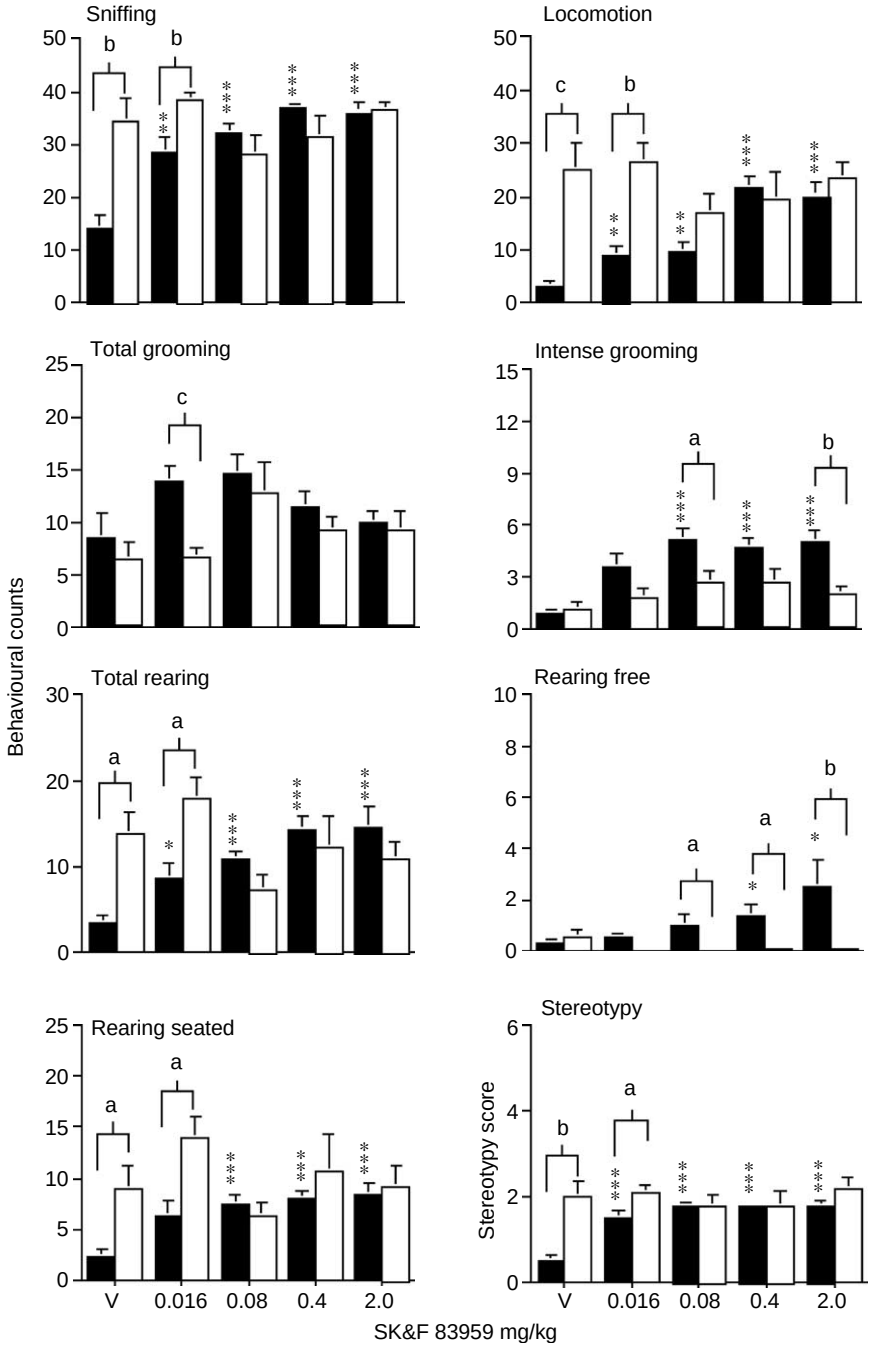


**Figure 5** Topographical effects of challenge with 0.016–2.0 mg/kg SK&F 83959 or vehicle (V) following 3 h of habituation. Data are mean behavioural counts ± SEM over a 60-min period, for sniffing, locomotion, total grooming, intense grooming, total rearing, rearing free, and rearing seated, with stereotypy scores, for wildtype ($n = 20$ females, filled columns) and congenic $D_{1A}$-null ($n = 20$ females, open columns) mice. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs* vehicle-treated control of the same genotype; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ between genotypes receiving the same dose.

emerged. Specifically, the expected habituation of sniffing, locomotion, rearing seated, and rearing to wall in wild types was substantially retarded in congenic $D_{1A}$ mutants. Thus, as these topographies of behaviour evidenced little or no diminution over later time periods, increases in locomotion and rearing seated relative to wildtypes became substantially greater than those over the initial exploratory period, with the emergence of marked increases in sniffing and rearing to wall that were not evident at all over initial exploration; this indicates how restricting behavioural assessment to a more limited time frame can obscure fundamental phenotypic effects. Conversely, rearing free, sifting, and grooming were reduced throughout habituation down to the levels ultimately attained by wildtypes; this indicates further how compositing distinct topographies of behaviour into an overall measure such as photobeam interruptions can obscure topographically specific aspects of phenotype.

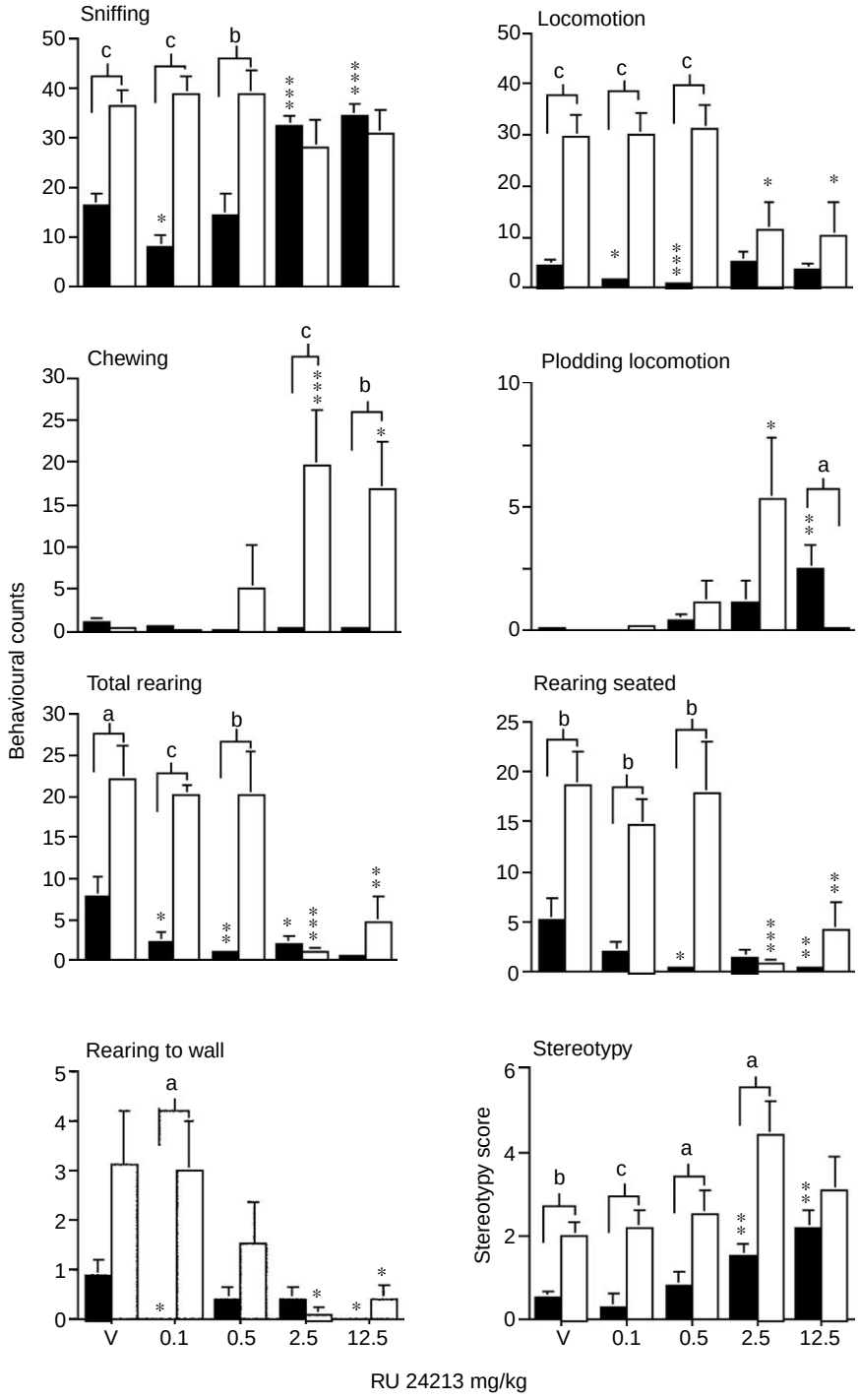


**Figure 6** Topographical effects of challenge with 0.1–12.5 mg/kg RU 24213 or vehicle (V) following 3 h of habituation. Data are mean behavioural counts ± SEM over a 60-min period, for sniffing, locomotion, chewing, plodding locomotion, total rearing, rearing seated, and rearing to wall, with stereotypy scores, for wildtype ($n = 20$ females, filled columns) and congenic $D_{1A}$-null ($n = 19$ females, open columns) mice. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs* vehicle-treated control of the same genotype; [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ between genotypes receiving the same dose.

There has been sustained concern (Gerlai, 1996; Crawley *et al*, 1997; Kelly *et al*, 1998; Phillips *et al*, 1999; Sandberg *et al*, 2000; Sanford *et al*, 2001; Waddington *et al*, 2001) that because of the mixed (129/Sv × C57BL/6) genetic background on which essentially all DA receptor subtype (and most other) knockouts have been constructed and examined to date, phenotypic effects might reflect not only the entity deleted but also variations in that genetic background between individual animals. More specifically, background genes from the parental strains may interact with the mutated gene, in a manner that could severely compromise the interpretation of the mutant phenotype. It is widely recognised that much of *in vivo* functional output derives from synergistic or epistatic allelic interactions (Kido *et al*,

2000) and thus heterogeneous disruption of such interactions, as a result of varying contributions from mouse strains that exhibit clear differences in behaviours, brain anatomy, and sensitivity to environmental perturbations, could lead to a differential phenotype depending on strain contribution, independent of the deletion; for example, such strain differences have been described for open field activity, learning and memory tasks, aggression, sexual and parental behaviours, acoustic startle and prepulse inhibition, the behavioural actions of ethanol, nicotine, cocaine, opiates, antipsychotics and anxiolytics, and gene expression profiling (Crawley *et al*, 1997; Sandberg *et al*, 2000; Lariviere *et al*, 2001).

There has been little in the way of systematically acquired data in the area of DA receptor subtype knockouts to address the substance or otherwise of these concerns. Kelly *et al* (1998) made phenotypic comparisons between $D_2$ mutants on a mixed (129/Sv × C57BL/6) background and incipient congenics following five backcrosses into 129/Sv or C57BL/6 strains. They found that phenotypic differences between wildtypes of each strain were more prominent than those between $D_2$ mutants and wild types within each strain; thus, motor function in this $D_2$ mutant line appeared to be influenced more by genetic background effects than by the absence of $D_2$ receptors. However, these studies involve only incipient congenicity because of the limited number of backcrosses applied, and no such data are yet available in relation to any other DA receptor knockout.

Here, backcrossing $D_{1A}$-null mice into a C57BL/6 background resulted in essential elimination (to $<0.005\%$) of any contribution from the 129/Sv component of their initially hybrid (mixed129/Sv × C57BL/6) background. In these congenic $D_{1A}$ mutants, progressive increases in individual topographies of behaviour over levels in matched wildtype counterparts were profoundly greater than those in $D_{1A}$ mutants on a mixed genetic background over their matched wildtype counterparts. There were generally similar extents of habituation in each wildtype group for behaviours such as locomotion, rearing to wall, grooming, and sifting, indicating little influence of genetic background (C57BL/6 *vs* 129/Sv × C57BL/6) on these topographies. However, habituation of behaviours such as sniffing and rearing free was slower in C57BL/6 than in 129/Sv × C57BL/6 wildtypes; while this might appear consistent with evidence suggesting reduced 'activity' in the 129/Sv strain (Rogers *et al*, 1999; Carter *et al*, 2001; Ralph *et al*, 2001), the present data indicate this to be a topographically specific rather than any generalised effect. More fundamentally, the extent of retardation of habituation in congenic $D_{1A}$ mutants was substantially greater than that in $D_{1A}$ mutants on a mixed background even on taking differences between wildtype profiles into account; thus, it was possible to demonstrate large time (0–370 min) × genotype (D1A-null *vs* wildtype) × background (congenic C57BL/6 *vs* mixed 129/Sv × C57BL/6) interactions on contrasting the present data with our previous data obtained in the same laboratory using identical experimental procedures (Clifford *et al*, 1998; Waddington *et al*, 2001).

In order to clarify the nature of this delayed phenotype in congenic $D_{1A}$ mutants, pharmacological studies were undertaken using selective $D_1$-like *vs* $D_2$-like agonists and antagonists. Following a 3-h period of habituation, to allow manifestation thereof, congenic $D_{1A}$ mutants receiving control injections evidenced a general heightening of nonstereotyped sniffing, locomotion, rearing seated, and rearing to wall, in a manner similar to that of their untreated counterparts over the same time period; variations in magnitude of effect most likely reflect some influence of the stress of vehicle injection. The selective $D_1$-like antagonist SCH 23390 readily reduced these behaviours in wildtypes, as expected, but was without effect to reduce this heightened phenotype in congenic $D_{1A}$ mutants. This would indicate firstly that such effects in wildtypes are indeed mediated via antagonism of $D_{1A}$ rather than of $D_{1B}$ or any distinct $D_1$-like receptor coupled to phosphoinositide hydrolysis (PI) (Mahan *et al*, 1990; Undie and Friedman, 1990; Undie *et al*, 1994); these findings elaborate to individual topographies of behaviour a previous finding that the action of SCH 23390 in wildtypes to reduce otherwise undifferentiated photobeam interruptions, and to induce catalepsy, is absent in $D_{1A}$-null mice on a mixed genetic background (Xu *et al*, 1994a,b). Secondly, this would indicate that these aspects of the congenic $D_{1A}$ mutant phenotype appear not to involve compensatory hyperfunction through $D_{1B}$ receptors, any distinct $D_1$-like receptor coupled to PI hydrolysis, or indeed 5-$HT_2$ receptors, all of which are blocked by SCH 23390 (Undie *et al*, 1994; Niznik *et al*, 2002).

The selective $D_2$-like antagonist YM 09151-2 also reduced these behaviours in wildtypes, as expected; however, although YM 09151-2 effected some reduction of this heightened phenotype in congenic $D_{1A}$ mutants, overall levels, particularly of locomotion, still remained elevated relative to wildtypes. This would firstly confirm that such effects of YM 09151-2 in wildtypes do not involve antagonism of $D_{1A}$ receptors; these findings elaborate to individual topographies of behaviour a previous finding that the action of the $D_2$-like antagonist haloperidol to induce catalepsy is retained in $D_{1A}$-null mice on a mixed genetic background (Moratalla *et al*, 1996). Secondly, this would indicate that this heightened phenotype in congenic $D_{1A}$ mutants appears not to involve, in any exclusive way, compensatory hyperfunction through $D_2$-like receptors.

SK&F 83959 shows high affinity and selectivity for $D_1$-like over $D_2$-like receptors, fails to stimulate adenylyl cyclase (AC), the defining characteristic of a $D_1$-like agonist, and indeed inhibits the stimulation of AC induced by DA, and thus satisfies criteria for classification as a $D_1$-like antagonist such as SCH 23390; yet its psychopharmacological profile in rodents and nonhuman primates is different from that of SCH 23390 and similar to that of AC-stimulating $D_1$-like agonists (Arnt *et al*, 1992; Deveney and Waddington, 1995; Gnanalingham *et al*, 1995; Waddington *et al*, 1998; Andringa *et al*, 1999; Niznik *et al*, 2002), and may involve a $D_1$-like receptor linked to PI (Panchalingham and Undie, 2001).

In wildtypes, SK&F 83959 induced grooming and particularly intense grooming, together with sniffing, locomotion, and rearing topographies in a nonstereotyped fashion, as expected (Clifford *et al*, 1999, 2001). The heightened phenotype in congenic $D_{1A}$ mutants could not be elevated further by SK&F 83959; conversely, induction of intense grooming was reduced, with induction of rearing

free essentially absent. Failure to influence the heightened phenotype in congenic $D_{1A}$ mutants mirrors that of SCH 23390, and suggests further that this aspect of phenotype is independent of $D_1$-like receptors. The re-emergence of grooming in congenic $D_{1A}$ mutants given high doses of SCH 23390, when these doses induce further reduction of grooming in wildtypes, might suggest the unmasking of an alternative, possibly $D_1$-like agonist effect of SCH 23390 that can be obscured in the course of its antagonism of $D_{1A}$ receptors (Niznik *et al*, 2002). Given the considerably greater magnitude of phenotypic effects in congenic $D_{1A}$ mutants relative to their counterparts on a mixed genetic background, it is difficult to compare these findings with previous findings in hybrid $D_{1A}$ mutants of reduced induction of photobeam interruptions by the AC-stimulating $D_1$-like agonist SK&F 81297 (Xu *et al*, 1994a) and relative preservation of topographical responsivity to SK&F 83959 and to the AC-stimulating $D_1$-like agonist A 68930 (Clifford *et al*, 1999).

The $D_2$-like agonist RU 24213 induced stereotyped sniffing and ponderous locomotion in wildtypes, with reductions in general locomotion, rearing topographies, and grooming, as expected (Clifford *et al*, 1999, 2001). The heightened phenotype in congenic $D_{1A}$ mutants was uninfluenced by lower doses, but affected by higher doses of RU 24213 as stereotyped behaviour was induced; at these higher doses, congenic $D_{1A}$ mutants evidenced a transition from some elevation in overall stereotyped sniffing and ponderous locomotion to stereotyped sniffing and chewing that were distinct from dose-dependent stereotypy of sniffing and ponderous locomotion in wildtypes. These findings would seem to indicate further that this heightened phenotype in congenic $D_{1A}$ mutants is independent of material $D_2$-like receptor involvement, although there appears to be some complex disruption of cooperative/synergistic and oppositional $D_1$-like:$D_2$-like interactions that are involved intimately in the regulation of individual topographies of DA-mediated behaviour (Waddington *et al*, 1994, 2001). No such effects were apparent when RU 24213 was given to $D_{1A}$ mutants on a mixed genetic background (Clifford *et al*, 1999).

In summary, repeated backcrossing into C57BL/6 over 14 generations, reducing the 129/Sv gene component to $<0.005\%$, revealed a $D_{1A}$-null phenotype qualitatively similar to but quantitatively much more profound than that seen on its original mixed (129/Sv $\times$ C57BL/6) background. Thus, in this instance modifier genes (Nadeau, 2001) appear to modulate critically the magnitude although not the fundamental nature of phenotypic effects. More generally, these results underscore the necessity for evaluating complex behavioural phenotypes of knockout mutations on well-defined, congenic backgrounds. Here, modifier genes exert a specific influence on the extent to which habituation processes are disrupted by developmental absence of $D_{1A}$ receptors to reveal a delayed phenotype over sustained assessment. These effects are topographically specific, and may involve compensatory processes consequent to the developmental absence of $D_{1A}$ receptors. Such processes appear not to involve other $D_1$-like receptors, and are at most only partially dependent on $D_2$-like receptors, hence, their neuronal basis remains to be specified.

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