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Comparative, Topographically-Based Evaluation of Behavioural Phenotype and Specification of D_1-Like:D_2 Interactions in a Line of Incipient Congenic Mice with D_2 Dopamine Receptor 'Knockout'

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Phenotypes were assessed topographically in mice lacking functional D_2 dopamine receptors ['knockouts'], using an ethologically based approach to assess all behaviours in the natural repertoire. D_2-null mice evidenced an ethogram characterised initially by modest reductions in locomotion and shifts in rearing topographies. Subsequently, topographies of behaviour habituated similarly for wildtypes and 'knockouts'. Following challenge with the D_2-like agonist RU 24213, both inhibition of rearing at a lower dose and induction of stereotyped sniffing and ponderous locomotion at higher doses were essentially absent in D_2-null mice. Following challenge with the D_1-like agonist A 68930, vacuous chewing was released in D_2-null mice. This topographical approach to phenotypic characterisation implicates: (i) the D_2 receptor in these D_2-like agonist effects and in oppositional D_1-like: D_2-like interactions; and (ii) the operation of material compensatory processes consequent to the developmental absence of D_2 receptors which are able to maintain ethological function under tonic, 'naturalistic' conditions but not under 'phasic' challenge.
[Neuropsychopharmacology 25:527–536, 2001]

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Received September 22, 2000; revised February 19, 2001; accepted February 26, 2001.

Online publication: 2/27/01 at www.acnp.org/citations/Npp 02270184.

KEY WORDS: *Dopamine D_2 receptor; D_2 'knockout'; Targeted gene deletion; Behavioural phenotype; Topographical assessment; Ethogram*

Members of the D_2-like dopamine (DA) receptor family [$D_{2L/S}$, D_3, D_4] (Bunzow et al. 1988; Giros et al. 1989; Sokoloff et al. 1990; Van Tol et al. 1991) constitute a series of proteins that are now recognised to play a fundamental role in the regulation of multiple aspects of mammalian psychomotor behaviour, both as independent entities and through functional interactions with their D_1-like [$D_{1A/1}$, $D_{1B/5}$] counterparts (Waddington et al. 1995, 2001; Missale et al. 1998). Although there are available both agonist and antagonist ligands which are

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/01/$–see front matter
PII S0893-133X(01)00246-9

highly selective for, and hence can discriminate readily *between*, these D_2-like vs. D_1-like receptor families, there are very few ligands which can yet discriminate materially *within* each of these families; thus, the distinct functions of individual D_2-like and D_1-like receptors are understood primarily at a 'family' level only (Waddington et al. 1998, 2001).

Over recent years, several groups have applied recombinant DNA technology to construct mice with targeted gene deletion ['knockout'] of individual dopamine receptor subtypes (Sibley 1999; Waddington et al. 2001), and three of these have concerned the D_2 subtype (Baik et al. 1995; Kelly et al. 1997, 1998; Jung et al. 1999). Although different genetic strategies were utilised, all three groups demonstrated comparable neurochemical evidence that functional D_2 receptors had been ablated. In contrast, although each of these D_2 knockout lines indicated a role for this receptor in maintaining aspects of normal behaviour, considerable inconsistencies were evident in relation to extent of deficit(s), including gross neurological phenotype. Baik et al. (1995) reported their D_2-null mouse line to evidence abnormalities of posture and gait, inability to reproduce, impairment in the rotarod task of sensorimotor co-ordination, and cataleptic-like behaviour with a marked reduction in observed line crossings and absence of vertical behaviour in an open field setting; conversely, Kelly et al. (1998) reported their D_2-null mouse line to evidence no comparable neurological phenotype, unaltered reproductive capacity, with reductions in horizontal and vertical movements in terms of photocell beam interruptions that were less prominent than those observed by Baik et al. (1995); yet Jung et al. (1999) reported their D_2 -null mice to share some of the features noted by Baik et al. (1995), including neurological impairment, transient bradykinesia and postural abnormalities, and greatly reduced levels of activity when examined in an open field, though their mice were fertile and capable of reproducing in accordance with the findings of Kelly et al. (1998).

It is important to note that each of these laboratories utilised a different genetic construct on a distinct genetic background, with examination of behavioural phenotype using diverse procedures, each of these factors being capable of influencing phenotype independent of the entity deleted (Crawley 1999; Gerlai 1999; Crabbe et al. 1999; Picciotto 1999; Waddington et al. 2001). Indeed, the studies of Kelly et al. (1998) suggest a material contribution from differences in genetic background to the diversity of findings between laboratories in relation to the behavioural phenotype of D_2 knockout. Recently, we have examined (Clifford et al. 2000) the initial cohort of D_2 knockouts constructed by Baik et al. (1995) using an approach that is ethologically-based in relation to strains of laboratory mice bred and housed over multiple generations under such conditions; this approach resolves all topographies of behaviour within the mouse repertoire and has been applied by us previously to define the *ethogram* of D_{1A} knockouts and to identify novel phenotypic features in terms of the 'sculpting' of behavioural topography over habituation from active exploration through to quiescence (Clifford et al. 1998, 1999). The phenotype for the D_2 knockout line of Baik et al. (1995) was characterised over initial exploration by modest but significant reductions in locomotion, grooming, rearing free and rearing to wall; rearing seated, sniffing, sifting and stillness were not altered. Individual elements of behaviour habituated similarly over a 6-h period for both genotypes, in the face of essential abolition of responsivity to D_2-like agonism; in contrast to the original report of Baik et al. (1995), we could not identify a prominence of 'parkinsonian-like' or other neurological components to the *ethogram* using these procedures (Clifford et al. 2000), as recently noted also by others using an alternative behavioural approach (Boulay et al. 1999a, 2000).

We have sought to resolve some aspects of these challenging phenotypic discrepancies by studying the incipient congenic line of D_2 knockout mice as described by Kelly et al. (1998) using a paradigm essentially identical to that utilised previously (Clifford et al. 2000) in studying the line described by Baik et al. (1995); thus, this is the first systematic comparison of these disputed phenotypes by an independent laboratory. Furthermore, we have contrasted spontaneous behavioural topography with responsivity to the well-established selective D_2-like agonist RU 24213 (Euvrard et al. 1980; Claudi et al. 1994; Waddington et al. 1995; Clifford et al. 1999) and applied the selective D_1-like agonist A 68930 (DeNinno et al. 1991; Daly and Waddington 1993; Waddington et al. 1995: Clifford et al. 1999) to probe for additional phenotypic effects at the level of D_1-like: D_2-like interactions.

METHODS

Transgenic Animals

The original F2 hybrid strain (129S2/SvPas × C57BL/6J) containing the mutated D_2 receptor allele was generated as reported previously (Kelly et al. 1997). In outline, the targeted gene deletion was constructed in 129S2/SvPas embryonic stem cells and male chimeras mated with C57BL/6J females to produce heterozygous mutants ($D_2^{+/-}$); homozygous mutants ($D_2^{-/-}$) and wildtype ($D_2^{+/+}$) littermates were identified among the progeny of heterozygous intermatings, using polymerase chain reaction (PCR) analysis of isolated tail DNA.

An incipient congenic D_2 line was established by backcrossing $D_2^{+/-}$ to wildtype C57BL/6 for five generations (Kelly et al. 1998). Incipient congenic $D_2^{+/-}$

mutants were transported to Dublin, where homozygous mutants ($D_2^{-/-}$) and wildtype ($D_2^{+/+}$) littermates were bred and genotyped by PCR among the progeny of heterozygous intermatings. They were housed in groups of 3–5 with food and water available *ad libitum*, and were maintained at 21 ± 1 °C on a 12 h/12 h (0700 on; 1900 off) light/dark schedule. Young adult mice from litters of the same generational age were used in behavioural assessments.

Behavioural Assessment

For evaluation of spontaneous behaviour, mice were removed from their home cage and placed individually in clear glass observation cages (36 × 20 × 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. 1998, 1999, 2000).

For this procedure, each of ten 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; locomotion (co-coordinated movement of all four limbs producing a change in location); total rearing (of any form); rearing from a sitting position (front paws reaching upwards with hind limbs on floor in sitting position); rearing free (front paws reaching upwards away from any cage wall while standing on hind limbs); rearing towards a cage wall (front paws reaching upwards onto or towards a cage wall while standing on hind limbs); sifting (sifting movements of the front paws through cage bedding material); grooming (of any form); intense grooming (grooming of the snout and face with the forepaws followed by vigorous grooming of the hind flank or anogenital region with the snout); vacuous chewing (chewing movements not directed onto any physical material); chewing (chewing movements directed onto physical material, i.e., cage bedding and/or fecal pellets, without consumption); eating (chewing with consumption); climbing (jumping onto cage top with climbing along grill in inverted or hanging position); and stillness (motionless, with no behaviour evident).

This cycle of assessment by behavioural checklist over a 15-min period (0–15 min) was repeated twice (20–35 min and 40–55 min); thereafter, 8 × 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. Thus, for evaluation of spontaneous behavioural topography each animal was observed according to the above protocol over a single test session of 370-min duration by an observer who was unaware of the genotype of each animal.

Evaluation of agonist-induced behaviour utilised procedures similar to those used for spontaneous behaviour; however, in these experiments animals were habituated to identical observation cages for a period of 3 h, to ensure that baseline activity was as low as possible before agonist challenge. Immediately following challenge with agonist or vehicle, each of ten randomly allocated mice was observed individually as above, with the 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). 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 activities; 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; and 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 session of 1 h. For evaluation of agonist-induced behaviour, mice were used on two occasions, separated by a drug-free interval of at least 1 week; on each occasion mice were allocated randomly to one of the various treatment groups.

All assessments were made by an observer unaware of treatment as well as genotype for each animal. 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 in accordance with Irish legislation and EU regulations for the care and use of experimental animals.

Drugs

RU 24213 (N-*n*-propyl-N-phenylethyl-*p*-3-hydroxyphenylethylamine; Hoechst-Marion-Roussel, France) was dissolved in distilled water; A 68930 ([1*R*,3*S*]-1-aminomethyl-5,6-dihydroxy-3-phenylisochroman; Abbott, USA) was dissolved in dilute acetic acid and made up to volume with distilled water. Both agents 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), for determination of *ethograms* for spontaneous behavioural topography over a phase of initial exploratory activity, the total 'counts' for each individual behaviour was determined as the number of 5-s observation windows in which a given behaviour was evident, summed over the initial 3 × 15-min (0–15, 20–35, 40–55 min) cycle periods, and expressed as means ± SEM. Data for individual behaviours were analysed using analysis of variance (ANOVA), following square-root transforma-

tion, to allow examination of interaction effects in the absence of non-parametric techniques for interaction terms. 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 ± SEM and analysed using repeated-measures ANOVA following square-root transformation.

For agonist-induced behaviour, the total 'counts' for each individual behaviour was determined as the number of 5-s observation windows in which a given behaviour was evident, summed over the initial 3 × 15-min (0–15, 20–35, 40–55 min) cycle periods, and expressed as means ± SEM; stereotypy scores were averaged over the 1 h period and expressed similarly. 'Counts' for individual behaviours in relation to agonist dose were analysed using ANOVA followed by Student's t-test or, in instances where data distribution deviated from normality, using the Kruskal-Wallis non-parametric ANOVA followed by Mann-Whitney U-test; stereotypy scores in relation to agonist dose were analysed using the Kruskal-Wallis non-parametric ANOVA followed by Mann-Whitney U-test.

RESULTS

General Parameters

On examining 25 [13 female, 12 male] D_2-null mice, mean body weight [19 ± 1 g; mean age 108 ± 7 days] was significantly reduced [−17%; $p = .001$] relative to 24 [15 female, 9 male] wildtype controls [23 ± 1 g; mean age 104 ± 7 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 ($n = 24$), D_2-null mice ($n = 25$) were characterised over the initial 1 h exploratory phase by reductions in locomotion [−25%; $F(1,45) = 15.89$; $p < .001$), rearing free [−43%; $F(1,45) = 8.45$; $p < .01$], and rearing to wall [−40%; $F(1,45) = 11.27$; $p < .01$] with an increase in rearing from a seated position [+30%; $F(1,45) = 14.24$; $p < .001$], in the absence of any significant effects of gender or of genotype × gender interactions; there were no significant differences in sniffing, total rearing or sifting, with only low levels of chewing, vacuous chewing, eating, climbing, and stillness in all groups without any apparent association to genotype (Figure 1).

Given the reduced bodyweight among D_2-null mice relative to wildtype controls, relationships between counts for individual elements of behaviour and weight were examined. Determination of Spearman coefficients over this phase indicated no significant correlations [all $p > .05$]: locomotion vs. bodyweight: $r_s = -0.09$ in D_2-null, $r_s = -0.001$ in wildtypes; rearing seated vs. bodyweight: $r_s = -0.25$ in D_2-null, $r_s = -0.11$ in wildtypes; rearing free vs. bodyweight: $r_s = -0.11$ in D_2-null, $r_s = -0.001$ in wildtypes; rearing to wall vs. bodyweight: $r_s = -0.21$ in D_2-null, $r_s = 0.01$ in wildtypes. Thus, phenotypic differences appeared unrelated to reduced bodyweight.

Ethogram of Spontaneous Behaviour Over Habituation Phase

These topographical shifts between behaviours in D_2-null mice ($n = 25$) were evident only over the initial exploratory phase; thereafter, behaviours habituated readily in a manner similar to that evident in wildtypes ($n = 24$), down to low baseline levels that, with the exception of locomotion, did not differ between genotypes (Figure 2); thus, repeated measures ANOVA revealed for locomotion a significant overall reduction in D_2-null mice relative to wildtypes [$F(1,45) = 5.04$; $p < .05$], with a significant overall reduction by time bins [$F(9,459) = 76.22$; $p < .001$] which did not differ by genotype [$F(9,459) = 1.07$; $p > .05$] in the absence of any time × genotype × gender interaction [$F(9,459) = 0.97$; $p > .05$].

Female mice exhibited higher overall levels of locomotion [$F(1,45) = 11.12$; $p < .01$], total rearing [$F(1,45) = 7.28$; $p < .01$] and rearing seated [$F(1,45) = 6.78$; $p < .05$] than did males, in the absence of any gender × genotype interactions, with no other effects of gender evident; in Figure 2, data are collapsed across gender, with intense grooming, vacuous chewing, eating, and climbing omitted because of values too low for meaningful analysis.

Ethogram of Responsivity to the Selective D_2-Like Agonist RU 24213

On examining 20 female D_2-null mice, mean body weight [18 ± 2 g; mean age 110 ± 4 days] was significantly reduced [−17%; $p < .001$] relative to 20 female wildtype controls [22 ± 1 g; mean age 108 ± 5 days].

Following challenge with the D_2-like agonist RU 24213 (0.1–12.5 mg/kg) (Figure 3), D_2-null mice evidenced essential abolition of the prominent induction of stereotyped sniffing, locomotion, and particularly of characteristic, ponderous locomotion (main effect of genotype, $p < .001$; main effect of treatment, $p < .001$; genotype × treatment, $p < .001$). Conversely, grooming, rearing free, rearing seated, and total rearing were reduced by RU 24213 in a manner that did not differ between the genotypes (main effects of treatment each $p < .001$, in the absence of any overall effect of genotype or genotype × treatment interaction); however, it was

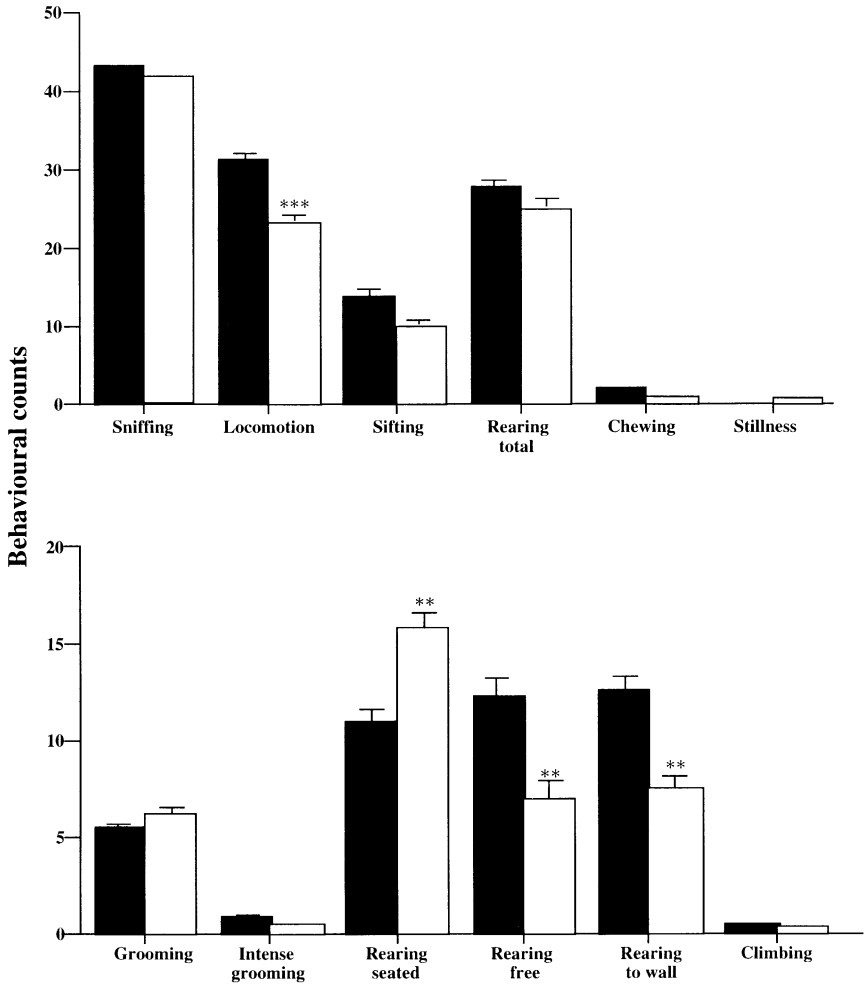

Figure 1. Behavioural counts for sniffing, locomotion, sifting, total rearing, chewing, stillness, grooming, intense grooming, rearing seated, rearing free, rearing to wall, and climbing in wildtype ($n = 24$; closed columns) vs. D_2-null ($n = 25$; open columns) mice. Data are mean counts ± SEM over a 1-h phase of initial exploratory activity. ***$p < .001$, **$p < .01$ vs. wildtype.

noted that the action of the lowest dose of RU 24213 to significantly reduce ($p < .05$) total rearing, rearing seated and stereotypy scores as seen in wildtypes was absent in D_2-null mice. Levels of intense grooming, chewing, vacuous chewing, eating, and climbing were too low for meaningful analysis.

Ethogram of responsivity to the selective D_1-like agonist A 68930

On examining 20 female D_2-null mice, mean body weight [15 ± 1 g; mean age 89 ± 7 days] was significantly reduced [−27%, $p < .001$] relative to 20 female wildtype controls [20 ± 1 g; mean age 96 ± 6 days].

Following challenge with the D_1-like agonist A 68930 (0.068–2.0 mg/kg) (Figure 4), D_2-null mice evidenced unaltered responsivity to the induction of sniffing, locomotion, rearing seated, total rearing and sifting, and particularly of characteristic grooming (main effect of treatment, $p < .001$; no effect of genotype or genotype × treatment interaction) and intense grooming. Conversely, induction of rearing free and rearing to wall was reduced (main effect of treatment, $p < .001$; effect of genotype, $p < .05$; no genotype × treatment interaction), whereas vacuous chewing to A 68930 was enhanced in D_2-null mice (main effect of treatment, $p < .01$; effect of genotype, $p < .001$; genotype × treatment, $p = .1$). Levels of chewing, eating, and climbing were too low for meaningful analysis.

DISCUSSION

This study seeks to compare two independently derived lines of D_2 receptor-deficient mice (Baik et al. 1995; Kelly et al. 1998) under essentially identical testing conditions, in an effort to differentiate those phenotypic differences which are the result of targeting this particular DA receptor subtype from those that are manifestations of other parameters (e.g., differences between strains and testing paradigms); it should be re-

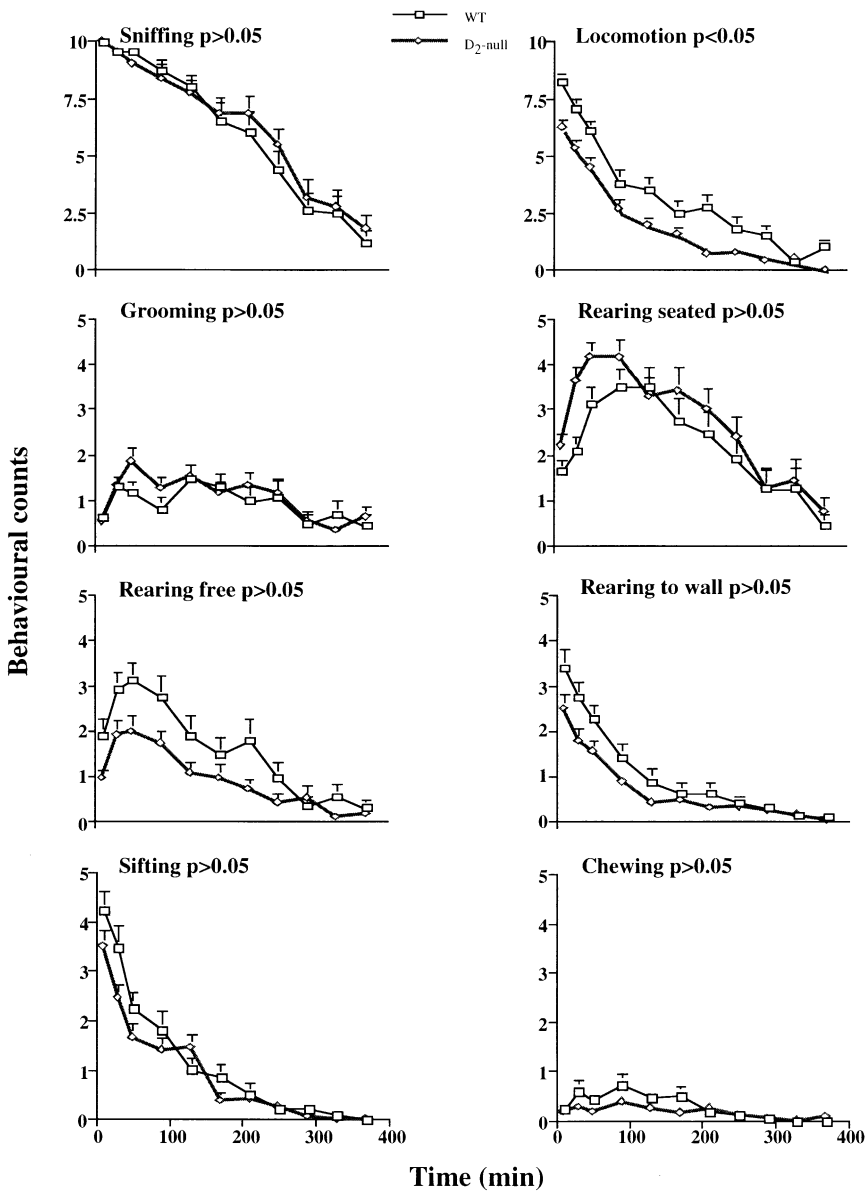

Figure 2. Behavioural counts for sniffing, locomotion, grooming, rearing seated, rearing free, rearing to wall, sifting, and chewing in wildtype ($n = 24$; open squares) vs. D_2-null ($n = 25$; open diamonds) mice. Data are mean counts ± SEM per 10-min period at indicated intervals over a 370-min phase of habituation, with overall effects of genotype on ANOVA indicated for each behaviour.

called that these two laboratories utilised different genetic constructs on a varying genetic background, with examination of behavioural phenotype using diverse procedures, each of these factors being capable of influencing knockout phenotype independent of the entity deleted (Picciotto 1999; Crawley 1999; Gerlai 1999; Crabbe et al. 1999; Waddington et al. 2001).

Using animals identical to those studied by Kelly et al. (1998), and adopting an ethologically-based approach to resolve and quantify all topographies of behaviour within the natural repertoire, we have identified the following *ethogram* for this line of incipient congenic D_2 knockout mice: over the phase of initial exploratory activity, there were modest but significant reductions in locomotion, rearing free, and rearing to wall, with heightened rearing from a seated position, in the absence of any gross motor phenotype. This phenotype is similar to that which we have reported recently (Clifford et al. 2000) for D_2 knockouts identical to those studied by Baik et al. (1995); indeed, juxtaposition of data in the present Figure 1 with comparable data in Figure 1 of Clifford et al. (2000) suggests that using the present assessment technique as applied similarly by the same observer reveals for these two D_2 knockout

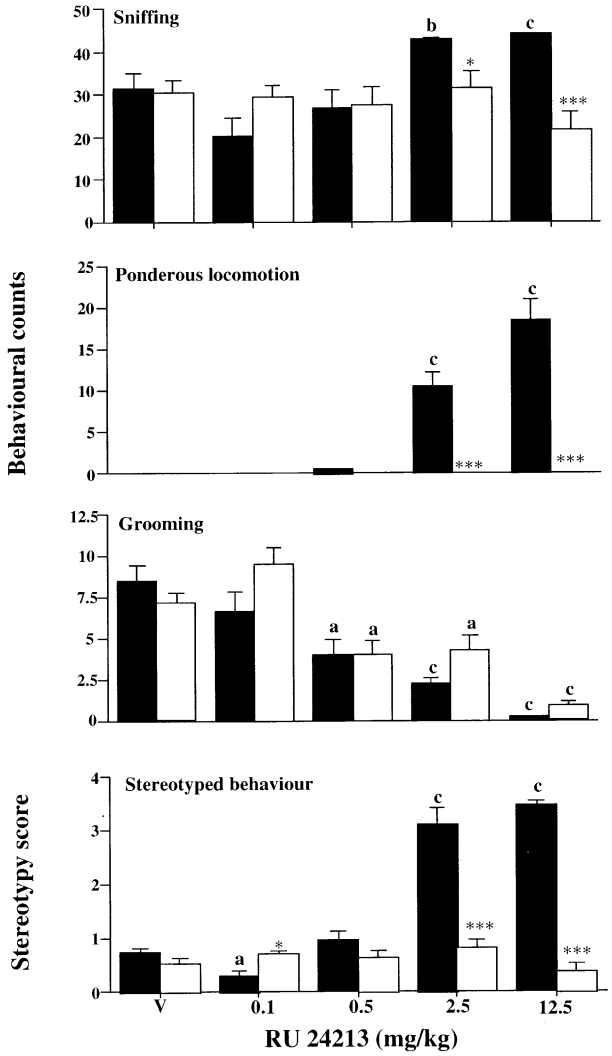

Figure 3. Behavioural counts for sniffing, ponderous locomotion, and grooming, with stereotypy scores, in wildtype (n = 20; closed columns) vs. D_2-null (n = 20; open columns) mice following challenge with 0.1–12.5 mg/kg RU 24213 or vehicle (V). Data are mean counts ± SEM over a 1-h period for n = 8 per group. ***$p < .001$, *$p < .05$ vs. wildtype receiving same treatment; $^c p < .001$, $^b p < .01$, $^a p < .05$ vs. vehicle-treated control of same genotype.

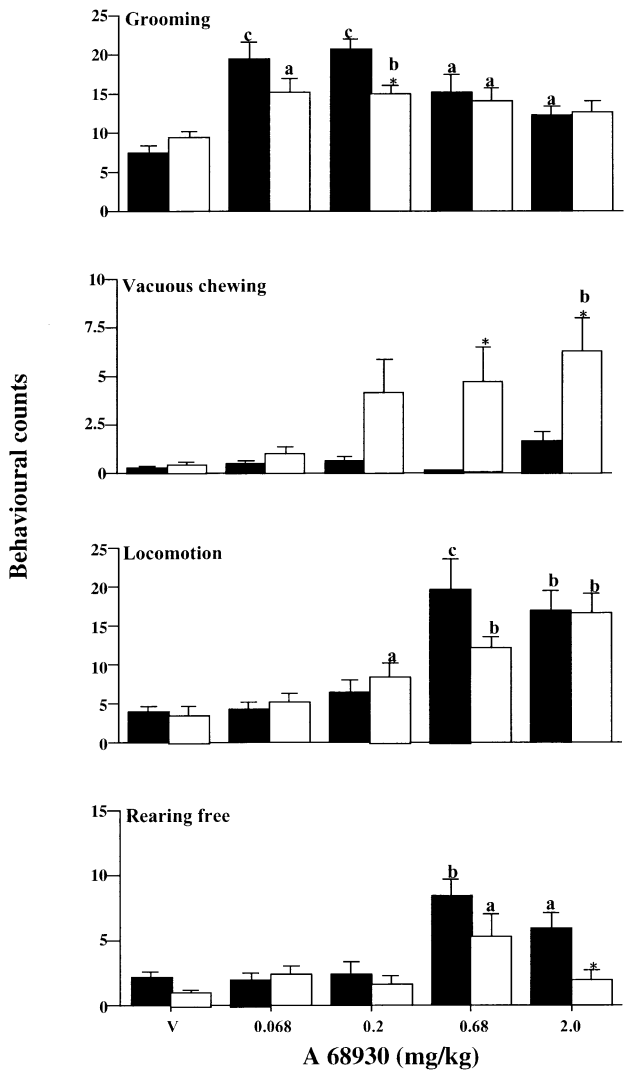

Figure 4. Behavioural counts for grooming, vacuous chewing, locomotion and rearing free, in wildtype (n = 20; closed columns) vs. D_2-null (n = 20; open columns) mice following challenge with 0.068–2.0 mg/kg A 68930 or vehicle (V). Data are mean counts ± SEM over a 1-h period for n = 8 per group. *$p < .05$ vs. wildtype receiving same treatment; $^c p < .001$, $^b p < .01$, $^a p < .05$ vs. vehicle-treated control of same genotype.

lines a generally comparable phenotype at this level of examination. This approach to phenotypic characterisation elaborates the findings of Kelly et al. (1998) by indicating reductions in horizontal and vertical photocell beam interruptions to relate specifically to ethologically-defined, exploratory locomotion and to individual topographies of rearing, particularly rearing free and rearing to wall; increases in rearing from a seated position may represent a shift in rearing topography from an exploratory to a sedentary form.

Continuing assessments over a subsequent 5-h period beyond the initial exploratory phase revealed ready habituation of behaviours, the extent of which did not further distinguish the present D_2-null and wildtypes. This habituation *ethogram* for the D_2 knockout line of Kelly et al. (1998) is also similar to that which we have described previously (Clifford et al. 2000) for the D_2 knockout line of Baik et al. (1995); indeed, juxtaposition of data in the present Figure 2 with comparable data in Figure 2 of Clifford et al. (2000) attests the similarity of phenotype for these two D_2 knockout lines at an additional level of behavioural examination. Also, these data emphasise a critical functional dissociation from the phenotype of D_{1A} knockouts, for which the habituation phase revealed the emergence of important

phenotypic differences which were not evident over initial exploration (Clifford et al. 1998); thus, the present data sustain and elaborate the notion that D_{1A} but not D_2 receptors interact with the neuronal substrate of habituation in 'sculpting' the changing topography of behaviour from initial exploration through to quiescence (Clifford et al. 1998, 2000; Waddington et al. 2001).

In their recent report, Kelly et al. (1998) offered evidence that genetic background effects make an important contribution to apparent phenotypic differences between their incipient congenic D_2 knockout line and that of Baik et al. (1995) on a mixed genetic background. In particular, on backcrossing their own mixed-background D_2 knockout both into 129S6/SvEvTac and into C57BL/6J strains, phenotypic differences between wildtypes of each strain were more prominent than those between knockouts and wildtypes within each strain; thus, motor function in this D_2 knockout line appeared to be influenced more by genetic background effects than by absence of D_2 receptors. These important findings complement a weight of evidence indicating an important contribution from genetic background in determining knockout phenotype (Gerlai 1999); indeed, we have reported recently that the phenotype of congenic D_{1A} knockouts, following 14 back-crosses into C57BL/6, shows material phenotypic differences (McNamara et al. 2001) from their counterparts having a mixed genetic background (Clifford et al. 1998).

In failing to identify here prominent phenotypic differences between these two D_2 knockout lines at this level of examination, the present data suggest that genetic background may be an important but not exclusive basis for such phenotypic differences when they are apparent. While after five back-crosses into C57BL/6J some influence of residual 129S2/SvPas background cannot be excluded, renewed attention is focussed also on issues of apparently similar but inherently heterogeneous methods and procedures used for assessing behavioural phenotype in differing laboratories (see Crabbe et al. 1999). Consequently, the present data substantiate the importance of having comparative studies of behavioural phenotype conducted by the same investigator using the same techniques, whether comparing two lines of the same putative knockout or the knockout of one receptor subtype with that of another subtype.

In the present study, typical stereotyped responsivity to the selective D_2-like agonist RU 24213 in wildtypes was essentially abolished in this line of D_2-null mice, in a manner similar to that which we have reported previously, using identical methods (Clifford et al. 2000), for the line of D_2 knockouts constructed by Baik et al. (1995). The consistency of these findings indicates a primary role for the D_2 receptor, rather than for D_3 or D_4 receptors, in this characteristic stimulatory action of D_2-like agonists. However, the present findings highlight the challenge posed previously (Clifford et al. 2000): if D_2-null mice are so profoundly unresponsive in terms of D_2-like agonist-induced stimulation of behaviour, how might it be explained that these animals show such modest reductions in the topography of spontaneous behaviour? For these reasons compensatory processes consequent to developmental absence of D_2 receptors, which are able to maintain in substance the topography of spontaneous behaviour over the present conditions, are to be suspected.

One methodological factor that might influence our finding of a generally comparable behavioural phenotype in the face of contradictory findings between the originators of these two D_2 knockout lines is the extent of stress placed on DAergic function. As we have argued elsewhere (Clifford et al. 2000), the paradigms of Baik et al. (1995), Kelly et al. (1998), and ourselves may have placed differing demands on DAergic systems. For example, Baik et al. (1995) examined their D_2 knockouts using a circular open field under conditions very different from the home cage, following a period of isolation rearing; conversely, we utilised an observation cage which contained usual bedding, under more 'naturalistic' conditions similar to the home cage. Compensatory processes might be more able to sustain function under 'tonic', ethologically relevant conditions, and less able to sustain function under more demanding, 'phasic' conditions. That D_3 and/or D_4 receptors might be able to subsume D_2-mediated functions is unlikely given their generally low density in D_2-abundant brain regions (Missale et al. 1998); furthermore, their genes appear to be expressed normally in D_2-null mice (Baik et al. 1995; Kelly et al. 1998), although Jung et al. (1999) report a temporally-specific upregulation of D_3 receptors in their line of D_2-null mice. The nature of such compensatory processes remains unclear but is deserving of considerable further study as an alternative route to reversing the sequelae of DAergic hypofunction (Waddington et al. 2001). Application of inducible gene knockout strategies in comparative studies with conventional knockout techniques may be one approach to addressing these challenges.

On examining further the dose-dependent effects of D_2-like receptor stimulation, a reduction in behaviour, particularly rearing, induced by a low dose of RU 24213 in wildtypes was markedly diminished in D_2 knockouts. Though more detailed dose-response studies are needed, these findings pertain to the controversy as to whether among D_2-like receptors it is the D_3 or D_2 receptor, or both, which mediates putative 'autoreceptor' or inhibitory postsynaptic functions (Levant 1997). Our finding here that the action of a low dose of RU 24213 to reduce specific topographies of behaviour is attenuated in D_2 knockouts, but unaltered in their D_3 counterparts (McNamara et al. 2000), as noted recently by Boulay et al. (1999a,b) using other D_2-like agonists, implicates the D_2 receptor in these effects; these findings are complementary to other behavioural and electrophysiological data

which attribute to the D_2 rather than the D_3 subtype a primary role as an 'autoreceptor' on DAergic neurones or as an inhibitory postsynaptic receptor (Mercuri et al. 1997; Clifford and Waddington 1998; L'hirondel et al. 1998; Koeltzow et al. 1998; Xu et al. 1999). Yet, unlike the abolition of stereotyped sniffing and ponderous locomotion in D_2 knockouts, the topographies of grooming, rearing free, rearing seated, and total rearing were reduced by higher doses of RU 24213 in a manner that did not differ between the genotypes; whether this effect involves D_3, some other DAergic receptor, or a non-DAergic site remains to be clarified.

In the control of DA-mediated behaviours D_2-like and D_1-like receptors do not function independently but, rather, are subject to critical D_1-like: D_2-like interactions. Typical D_2-like-initiated behaviours such as stereotyped sniffing and locomotion are regulated in a co-operative/synergistic manner by tonic or phasic activity through D_1-like receptors; conversely, atypical behaviours appear to be regulated in an oppositional manner such that vacuous chewing has its genesis in tonic or phasic activity through D_1-like receptors but is released/enhanced by reduction in DAergic activity through D_2-like receptors (Waddington et al. 1994, 1995, 1998). However, the involvement of individual family members in these effects is poorly understood.

The present finding of enhanced vacuous chewing to the D_1-like agonist A 68930 in D_2-null mice is consistent with the regulation of this behaviour through interactions of the D_1-like family with D_2 rather than with D_3 or D_4 receptors. However, minimal effects of D_2 knockout on D_1-like agonist-induced grooming and other behaviours would not exclude an involvement of putative D_1-like: D_3 interactions in the regulation of this and other behaviours; indeed, evidence has been offered that the D_3 receptor inhibits co-operative/synergistic D_1-like: D_2-like interactions in terms of otherwise undifferentiated photocell beam interruptions (Xu et al. 1997).

It should be noted that the present work and our previous studies (Clifford et al. 1998,1999, 2000) indicate that individual topographies of behaviour can be differentially regulated by individual DA receptor subtypes and the interactions between them. Thus, the compositing of heterogeneous behaviours using, for example, photocell beam approaches, has the potential to obscure important functional correlates (Waddington et al. 2001). It remains a paradox that the molecular technology, which isolates individual DA receptor subtypes, has been so often assessed functionally by techniques which composite individual topographies of behaviour.

ACKNOWLEDGMENTS

These studies were supported by the Higher Education Authority of Ireland, a Galen Research Fellowship from the Irish Brain Research Foundation, and the Research Committee of the Royal College of Surgeons in Ireland [JJC and JLW], and by DA12062 [DKG]. We thank Abbott for A 68930 and Hoechst-Marion-Roussel for RU 24213.

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