Comparative Phenotypic Resolution of Spontaneous, D_2-Like and D_1-Like Agonist-Induced Orofacial Movement Topographies in Congenic Mutants With Dopamine D_2 vs. D_3 Receptor "Knockout"

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ABSTRACT Using a novel system, the role of D_2-like dopamine receptors in distinct topographies of orofacial movement was assessed in mutant mice with congenic D_2 vs. D_3 receptor knockout, and compared with findings in D_{1A} mutants. Under spontaneous conditions, D_2 mutants evidenced increased vertical jaw movements and unaltered horizontal jaw movements, with reductions in tongue protrusions and incisor chattering; in D_3 mutants, only incisor chattering was reduced. Given previous evidence that D_{1A} mutants show reduced horizontal but not vertical jaw movements, this indicates that apparent oppositional D_1-like:D_2-like interactions in the regulation of composited jaw movements may in fact reflect the independent actions of D_2 receptors to inhibit vertical jaw movements and of D_{1A} receptors to facilitate horizontal jaw movements. Effects of the D_2-like agonist RU 24213 to exert greater reduction in horizontal than in vertical jaw movements were not altered prominently in either D_2 or D_3 mutants. The D_1-like agonists A 68930 and SK&F 83959 induced vertical jaw movements, tongue protrusions, and incisor chattering; induction of tongue protrusions by A 68930 was reduced in D_2 mutants. D_2 receptors exert topographically specific regulation of orofacial movements in a manner distinct from their D_{1A} counterparts, while D_3 receptors exert only minor regulation of such movements. **Synapse 51:71–81, 2004.**

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INTRODUCTION

In accordance with their origins in molecular biology rather than in classical functional considerations, attributing specific roles to individual members of the D_1-like (D_{1A}/D_1, D_{1B}/D_5) and D_2-like ($D_{2L/S}$, D_3, D_4) families of dopamine (DA) receptors (Missale et al., 1998) in the regulation of behaviour remains problematic (Waddington et al., 1995, 2001). While they appear to exert important roles in the regulation of orofacial movements, with a particular focus on how these are regulated by the D_1-like family, the D_2-like family can also influence such processes, both independently and especially via D_1-like:D_2-like interactions (Rosengarten et al., 1983, 1986; Murray and Waddington, 1989; Collins et al., 1991; Waddington et al., 1994, 1998; Niznik et al., 2002).

In contrast to the availability of both agonists and antagonists which are highly selective for, and hence can discriminate readily *between,* these D_1-like vs. D_2-like receptor families, there are very few agents which can discriminate materially *within* each of these families; thus, the functions of D_1-like and D_2-like receptors are understood primarily at a "family" level only (Waddington et al., 1998, 2001). Over recent years, recombinant DNA techniques have been applied by several groups to construct mice with targeted gene deletion (knockout) of individual DA receptor subtypes (Sibley, 1999; Waddington et al., 2001). Yet their potential to clarify the roles of individual DA receptor subtypes in regulating orofacial movements remains to be realised; indeed, systematic assessment of such movements is only now being undertaken even in normal mice because of practical issues: mice are considerably smaller than rats and their orofacial movements more rapid, making for problems in assessment. These difficulties are exacerbated by considerable controversy, based primarily on data in rats, as to how orofacial movements should be defined phenomenologically and resolved empirically; generic terms such as "vacuous chewing" enjoy widespread usage despite uncertainty as to their relevance at a physiological level (Waddington, 1990; Waddington et al., 1998; Tomiyama et al., 2001, 2002).

For these reasons, we recently developed a novel system combined with a physiologically based approach to categorisation and quantification for the assessment of orofacial movement topography in mice (Tomiyama et al., 2001). This has been applied to describe the phenotype of orofacial movements and topographical responses to D_2-like and D_1-like agonists in mice with congenic D_{1A} receptor knockout (Tomiyama et al., 2002). We now describe the application of this technique to characterise topographically, in a complementary, comparative manner, the phenotype of orofacial movements and topographical responses to the D_2-like agonist RU 24213 and the D_1-like agonists A 68930 and SK&F 83959 in mice with incipient congenic D_2 receptor knockout and congenic D_3 receptor knockout.

MATERIALS AND METHODS

Animals

The original F2 hybrid strain (129/Sv × 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 129/Sv embryonic stem cells and male chimaeras 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 similar PCR of isolated tail DNA among the progeny of heterozygous intermatings (Clifford et al., 2001).

The original F2 hybrid strain (129/Sv × C57BL/6) containing the mutated D_3 receptor allele was generated as reported previously (Accili et al., 1996). In outline, the targeted gene deletion was constructed in 129/Sv embryonic stem cells and male chimaeras mated with C57BL/6 female to produce heterozygous mutants ($D_3^{+/-}$); homozygous mutants ($D_3^{-/-}$) and wildtypes ($D_3^{+/+}$) were identified among the progeny of heterozygous intermatings using PCR analysis of isolated tail DNA. To establish an essentially congenic line of D_3 knockouts, heterozygous mutants of this hybrid (129/Sv × 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 (McNamara et al., 2002). Analysis of isolated tail DNA was used similarly to identify congenic, homozygous mutants and wildtypes among the progeny of heterozygous intermatings.

Animals were housed in groups of 3–5 with food and water available ad libitum and were maintained at 21.0 ± 0.1°C on a 12/12 h (07.00 on; 19.00 off) light/dark schedule. Young adult mice from litters of the same generational age were used in behavioural assessments. These studies were approved by the Research Committee of the Royal College of Surgeons in Ireland and were conducted under license from the Department of Health in accordance with Irish legislation and European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

Restrictor system

As described previously (Tomiyama et al., 2001), the system consisted of a "restrictor," by which mice were lightly restrained around the neck by a clear perspex collar attached to a horizontal platform; this allowed visual observation to be focused onto the orofacial region with minimal disturbance to movements other than locomotion, rearing, and grooming. Circular collars were composed of two semicircular elements: one fixed to the platform and constituting a trough into which the neck was positioned; the other, inserted from above, completed light enclosure of the neck. Both the diameter of the collar and its height above the platform were adjustable according to body size, to allow a comfortable posture to be maintained. A piece of absorbent paper was spread over the platform of the restrictor. To facilitate observation of the orofacial region, small mirrors were fixed in inclined positions just under the snout of each mouse and lighting directed appropriately to illuminate the mouth. For each experimental session, five mice were placed individually into identical "restrictors," each separated by cardboard dividers to minimise visual and auditory disruption. The observer viewed each animal through slits in a cardboard screen in front of the array of "restrictors"; these slits were positioned optimally in relation to the mouth, mirrors, and illumination.

Assessment of orofacial movement topography

On the basis of the natural repertoire of behaviours of the mouse at an ethological level, together with dental physiology, orofacial movement topography was categorised into the following four elements: vertical jaw movements, horizontal (lateral) jaw movements, tongue protrusions, and chattering (high-frequency rhythmical jaw movements with incisor tapping) (Tomiyama et al., 2001); general head movements and vibrissae movements were also recorded.

A rapid time-sampling behavioural checklist technique, used previously to resolve the topography of general exploratory and DA agonist-induced behaviour in knockout mice in an ethologically based, unrestricted paradigm (Clifford et al., 1998, 1999, 2000, 2001; Ross et al., 2000; McNamara et al., 2002, 2003), was applied similarly to resolve the topography of orofacial movement (Tomiyama et al., 2001, 2002): each of five mice was observed sequentially for 5-sec periods at 25-sec intervals; for each mouse, the presence or absence of each individual element (occurring alone or in any combination) was determined in each of the 5-sec periods. For assessment of spontaneous orofacial movement topography and its habituation profile, assessments commenced immediately after placement in restrictors and continued for 30-min periods over a total duration of 210 min; mice were used on a single occasion only. For assessment of orofacial movement topography in challenge studies, mice were habituated to restrictors for a period of 3 h before administration of drug or vehicle, with assessments beginning thereafter over a total duration of 1 h; mice were used on two occasions only, separated by a drug-free interval of at least 1 week, with random allocation to one of the various treatments in each instance. All observations were made by a dentist (KT) experienced also in rodent psychopharmacology who was unaware of genotype and treatment given to each animal.

Drugs

The selective D_2-like agonist RU 24213 (*N*-*n*-propyl-*N*-phenyl-*p*-3-hydroxyphenylethylamine; Hoechst-Marion-Roussel, France) was dissolved in distilled water. The D_1-like agonist A 68930 (([1*R*,3*S*]-1-aminomethyl-5,6-dihydroxy-3-phenylisochroman; Abbott Laboratories, North Chicago, IL, USA) was dissolved in dilute acetic acid and made up to volume with distilled water. The selective D_1-like agent SK&F 83959 (3-methyl-6-chloro-7,8-dihydroxy-1-[3-methyl-phenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine; Research Biochemicals International, Natick, MA / NIMH Chemical Synthesis Program, Bethesda, MD, USA) was dissolved in distilled water. Drugs or vehicle were injected subcutaneously into the flank in a volume of 2 ml/kg.

Data analysis

For determination of habituation profiles of spontaneous orofacial movement topographies, total counts for each individual element were summed separately over the following periods: 0–30, 60–90, 120–150, 180–210 min. In drug challenge studies, these counts were summed over 0–60 min after the habituation period and subsequent drug administration. Data were expressed as mean ± SEM and analysed using repeated-measures analysis of variance (ANOVA) after square-root transformation in the absence of appropriate non-parametric techniques for interaction terms. Individual group comparisons were then made using Student's *t*-test or Kruskal-Wallis nonparametric one-way ANOVA and Mann-Whitney *U*-test (McNamara et al., 2002, 2003; Ross et al., 2000; Tomiyama et al., 2001, 2002).

RESULTS

General parameters: spontaneous behaviour

On examining 40 (20 male, 20 female) incipient congenic D_2 mutants for spontaneous orofacial topography, mean body weight (18 ± 1 g; mean age 114 ± 4 days) was significantly reduced (–22%; $P < 0.001$) relative to 38 (18 male, 20 female) wildtype controls (23 ± 1 g; mean age 116 ± 6 days). On qualitative inspection of posture, reactivity to handling, and general activity, no gross motor phenotype was apparent. These findings were as noted previously for D_2 mutants of this incipient congenic line (Kelly et al., 1997, 1998; Clifford et al., 2001).

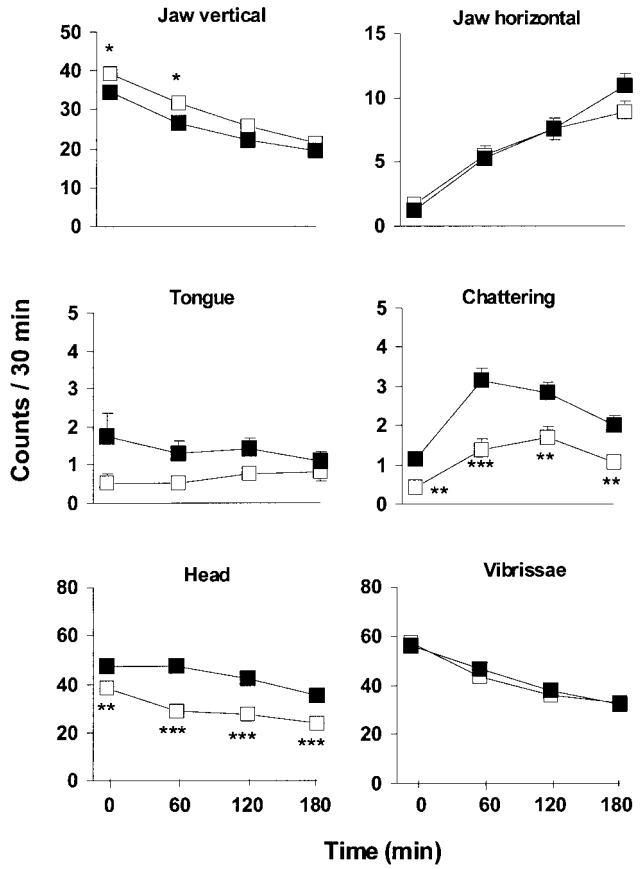

Fig. 1. Phenotype of spontaneous orofacial movement topographies in wildtypes (n = 38, filled squares) and incipient congenic D_2 mutants (n = 40, open squares). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over 30-min periods beginning at 0, 60, 120, and 180 min after commencing observations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. wildtypes.

On examining 20 (10 male, 10 female) congenic D_3 mutants for spontaneous orofacial topography, mean body weight (23 ± 1 g; mean age 113 ± 5 days) did not differ from 20 (10 male, 10 female) wildtype controls (22 ± 1 g; mean age 109 ± 6 days). On qualitative inspection of posture, reactivity to handling, and general activity, no gross motor phenotype was apparent. These findings were as noted previously for D_3 mutants both on a mixed (Accili et al., 1996; Xu et al., 1997) and on a congenic (McNamara et al., 2002) genetic background.

Spontaneous orofacial topography over habituation

D_2 mutants

In wildtypes, vertical jaw movements were initially prominent but declined subsequently over the habituation period (Fig. 1). Congenic D_2 mutants evidenced a small overall increase in such movements, which habituated similarly (overall effect of genotype, $F(1,74) = 4.71$, $P < 0.05$; no genotype × time interaction). Conversely, horizontal jaw movements occurred initially at a low level but increased markedly thereafter in wildtypes. This profile was unaltered in D_2 mutants (no overall effect of genotype or time × genotype interaction). Tongue protrusions and incisor chattering occurred at relatively low levels throughout habituation in wildtypes. These movements were reduced in D_2 mutants (overall effects of genotype: tongue protrusions, $F(1,74) = 12.10$, $P < 0.001$; incisor chattering, $F(1,74) = 30.28$, $P < 0.001$; no time × genotype interactions).

General head movements were initially prominent but then declined over habituation in wildtypes. In D_2 mutants these movements were reduced and habituated more rapidly over the early period of assessment (overall effect of genotype, $F(1,74) = 27.22$, $P < 0.001$; time × genotype interaction, $F(3,222) = 4.95$, $P < 0.01$). General movements of the vibrissae were initially prominent and declined over the habituation period. This profile was unaltered in D_2 mutants (no overall effect of genotype or time × genotype interaction).

In relation to gender, tongue protrusions and incisor chattering were more prominent in males of both genotypes (overall effects of gender: tongue protrusions, $F(1,74) = 10.84$, $P < 0.01$; incisor chattering, $F(1,74) = 4.14$, $P < 0.05$; no genotype × gender interactions), while the rate of habituation of general vibrissae movements was more rapid in males of both genotypes (time × gender interaction, $F(3,222) = 3.78$, $P < 0.05$; no time × gender × genotype interaction). No other effects of gender were encountered.

D_3 mutants

In wildtypes, vertical jaw movements, horizontal jaw movements, and tongue protrusions evidenced habituation profiles (Fig. 2) similar to those described above, with this profile not altered materially in congenic D_3 mutants (no overall effects of genotype or time × genotype interactions) other than some transient reduction in horizontal jaw movements. The habituation profile of incisor chattering in wildtypes was also similar to that described above, while in D_3 mutants the level of this movement was decreased (overall effect of genotype, $F(1,36) = 9.48$, $P < 0.01$). General head and vibrissae movements were initially prominent and declined over habituation in wildtypes, as described above. This profile was not altered materially in D_3 mutants (no overall effects of genotype or time × genotype interactions), other than some transient increase in head movements.

In relation to gender, vertical jaw movements were less prominent in males than in females for both genotypes (overall effect of gender, $F(1,36) = 9.14$, $P < 0.01$; no genotype × gender interaction), while horizontal jaw movements showed more rapid increase with time in males than in females for both genotypes (no overall

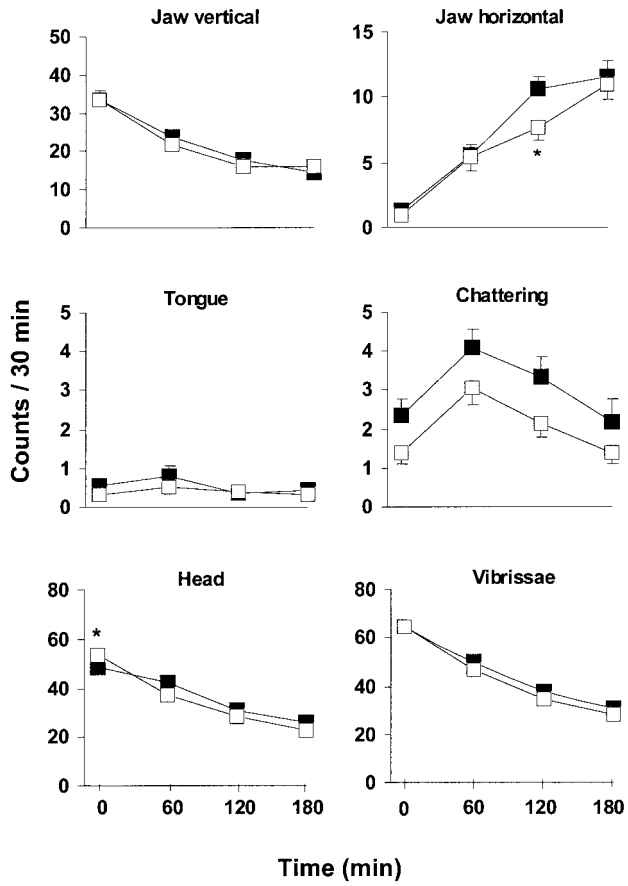

Fig. 2. Phenotype of spontaneous orofacial movement topographies in wildtypes (n = 20, filled squares) and congenic D_3 mutants (n = 20, open squares). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over 30-min periods beginning at 0, 60, 120, and 180 min after commencing observations. *$P < 0.05$ vs. wildtypes.

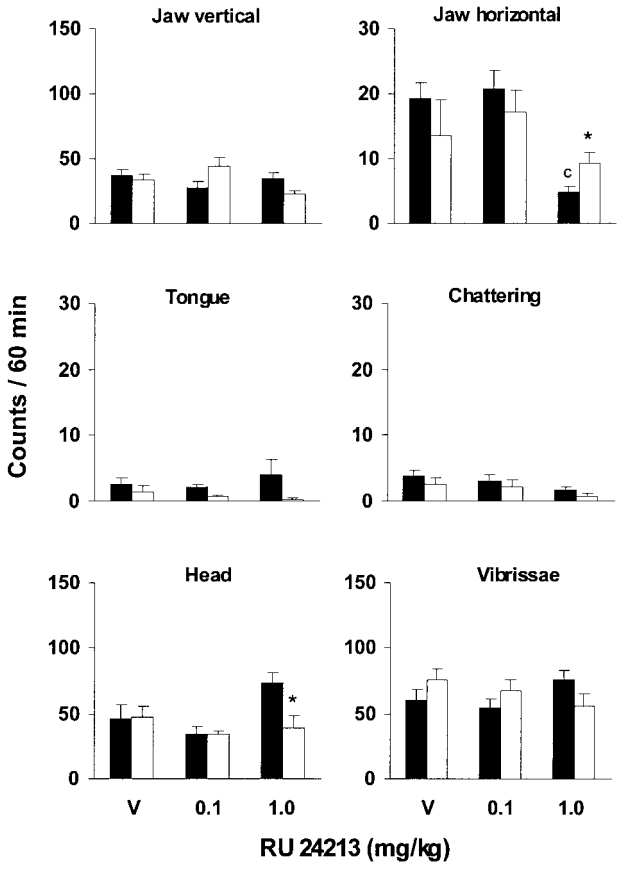

Fig. 3. Phenotype of orofacial movement topographies in wildtypes (n = 8, filled columns) and incipient congenic D_2 mutants (n = 5–6, open columns) following challenge with 0.1–1.0 mg/kg RU 24213 or vehicle (V). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over a 60-min period. °$P < 0.001$ vs. vehicle; *$P < 0.05$ vs. wildtypes receiving same dose.

effect of gender or genotype × gender interaction; time × gender interaction, $F(3,108) = 4.43$, $P < 0.01$; no time × gender × genotype interaction). The time course of incisor chattering in males relative to females differed marginally between the genotypes (no time × genotype interaction; time × genotype × gender interaction, $F(3,108) = 2.87$, $P < 0.05$). Apart from the isolated instances noted above, there were no other overall effects of gender, genotype × gender, or time × genotype × gender interactions.

General parameters: RU 24213 challenge

On examining eight male incipient congenic D_2 mutants for orofacial topography in response to RU 24213, mean body weight (15 ± 1 g; mean age 86 ± 7 days) was reduced (–38%; $P < 0.001$) relative to 12 male wildtype controls (24 ± 1 g; mean age 91 ± 7 days).

On examining 10 male congenic D_3 mutants for orofacial topography in response to RU 24213, mean body weight (23 ± 1 g; mean age 89 ± 2 days) did not differ from 10 male wildtype controls (23 ± 1 g; mean age 101 ± 5 days); the age of D_3 mutants was slightly less than wildtype counterparts (–12%, $P < 0.05$).

RU 24213-induced orofacial topography D_2 mutants

In wildtypes, RU 24213 did not exert any consistent effect on vertical jaw movements (Fig. 3). In congenic D_2 mutants this profile was not altered materially, although there were some subtle differences between the genotypes in relation to dose (no overall effects of dose or genotype; dose × genotype interaction, $F(1,36) = 7.80$, $P < 0.01$). Horizontal jaw movements were reduced by increasing doses of RU 24213. This effect did not differ prominently between the genotypes (overall effect of dose, $F(1,36) = 6.61$, $P < 0.02$; no overall effect of genotype or dose × genotype interaction), although it appeared marginally attenuated in D_2 mutants at the higher dose of RU 24213. Tongue protrusions were not influenced by RU 24213. These movements were reduced among D_2 mutants relative to wildtypes in a manner unrelated to treatment (no

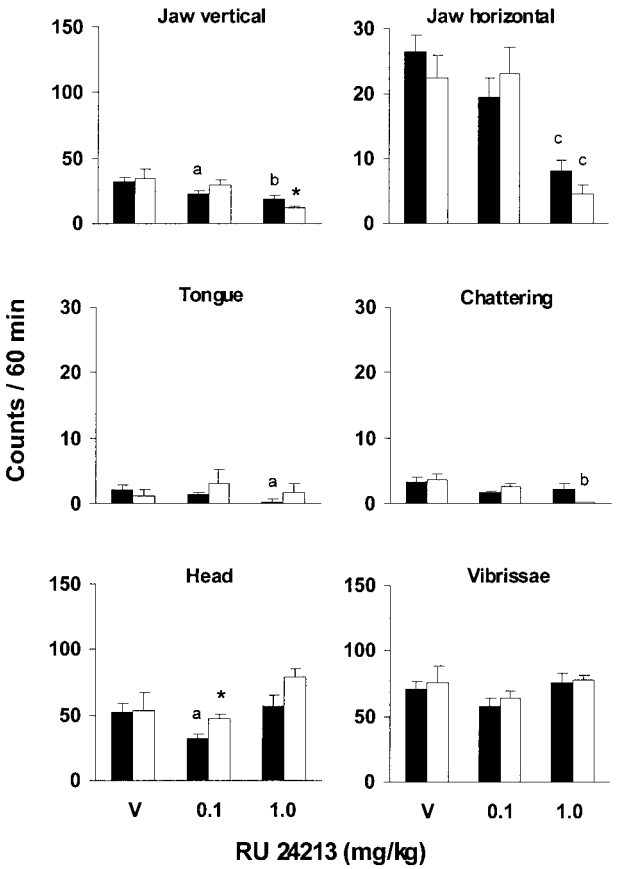

Fig. 4. Phenotype of orofacial movement topographies in wildtypes (n = 6–8, filled columns) and congenic D_3 mutants (n = 6–7, open columns) following challenge with 0.1–1.0 mg/kg RU 24213 or vehicle (V). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over a 60-min period. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs. vehicle; $^{*}P < 0.05$ vs. wildtypes receiving same dose.

overall effect of dose or dose × genotype interaction; overall effect of genotype, F(1,36) = 5.26, $P < 0.05$). Incisor chattering was not influenced materially by RU 24213 (no overall effects of dose or of genotype, or dose × genotype interaction). RU 24213 exerted only subtle, biphasic effects on head movements, which did not differ materially between the genotypes (overall effect of dose, F(1,36) = 4.17, $P < 0.05$; no overall effect of genotype or dose × genotype interaction), although the effect of the higher dose of RU 24213 appeared marginally attenuated in D_2 mutants. There were no alterations in vibrissae movements (no overall effects of dose or of genotype, or dose × genotype interaction).

D_3 mutants

In wildtypes, RU 24213 reduced both vertical and particularly horizontal jaw movements, with a low baseline level of tongue protrusions being reduced further (Fig. 4). In congenic D_3 mutants this profile was not altered materially (overall effects of dose: vertical jaw movements, F(2,34) = 15.29, $P < 0.001$; horizontal jaw movements, F(2,34) = 28.76, $P < 0.001$; no effects of genotype or dose × genotype interactions), other than a marginally greater effect on vertical jaw movements in those receiving the highest dose of RU 24213. Incisor chattering was reduced by RU 24213, somewhat more prominently in D_2 mutants than in wildtypes (overall effect of dose, F(2,34) = 8.57, $P < 0.001$; no overall effect of genotype; dose × genotype interaction, F(2,34) = 3.76, $P < 0.05$). RU 24213 exerted a biphasic effect on head movements, which did not differ materially between the genotypes (overall effect of dose, F(2,34) = 6.05, $P < 0.01$; no overall effect of genotype or dose × genotype interaction), although the action of the lower dose of RU 24213 to reduce such movements appeared marginally attenuated in D_3 mutants. There were no alterations in vibrissae movements (no overall effects of dose or of genotype, or dose × genotype interaction).

General parameters: A 68930 challenge

On examining 10 male incipient congenic D_2 mutants for orofacial topography in response to A 68930, mean body weight (20 ± 1 g; mean age 168 ± 12 days) was reduced (–29%; $P < 0.001$) relative to 10 male wildtype controls (28 ± 1 g; mean age 169 ± 10 days). Limited availability precluded A 68930 challenge studies in D_3 mutants.

A 68930-induced orofacial topography

In wildtypes, A 68930 readily induced vertical jaw movements (Fig. 5). This response was unaltered in incipient congenic D_2 mutants (overall effect of dose, F(2,32) = 26.32, $P < 0.001$; no overall effect of genotype or dose × genotype interaction). There was no induction of horizontal jaw movements in either genotype (no overall effects of dose or of genotype, or dose × genotype interaction), with a marginal reduction in baseline levels in vehicle-treated mutants. A 68930 readily induced tongue protrusions in wildtypes. This effect was substantially diminished in D_2 mutants at the higher dose of A 68930 (overall effect of dose, F(2,34) = 21.21, $P < 0.001$; overall effect of genotype, F(1,34) = 5.64, $P < 0.05$; dose × genotype interaction, F(2,34) = 6.26, $P < 0.01$). While A 68930 readily induced incisor chattering in both genotypes, levels were slightly reduced in D_2 mutants relative to wildtypes across all treatment groups (overall effect of dose, F(2,34) = 36.22, $P < 0.001$; overall effect of genotype, F(1,34) = 4.74, $P < 0.05$; no dose × genotype interaction). There was no consistent induction of general head movements by A 68930 in either genotype, although at the higher dose these were reduced in D_2 mutants (no overall effect of dose; overall effect of genotype, F(1,34) = 6.69, $P < 0.05$; dose × genotype interaction, F(2,34) = 5.48, $P < 0.01$). There were no effects on movements of the vibrissae.

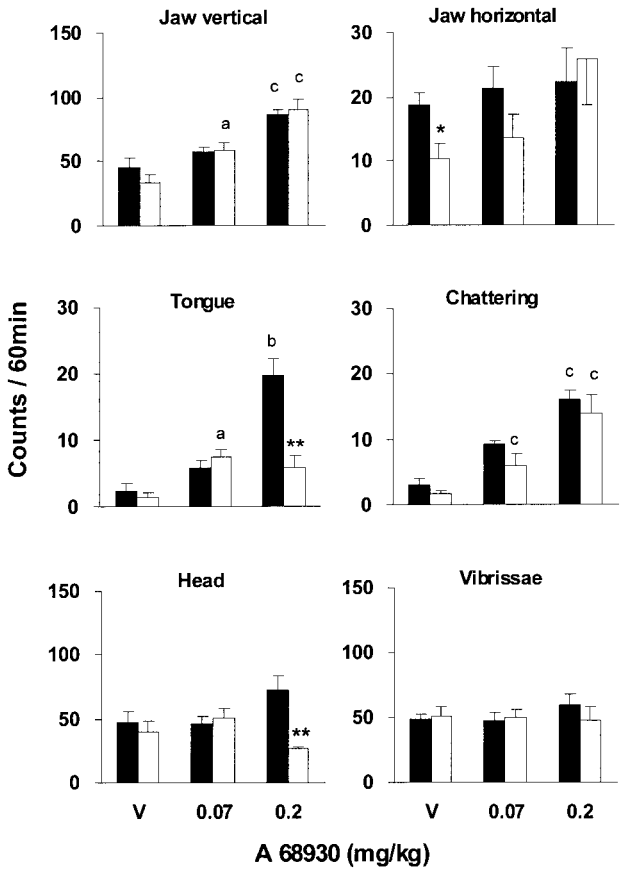

Fig. 5. Phenotype of orofacial movement topographies in wildtypes (n = 6–7, filled columns) and incipient congenic D_2 mutants (n = 6–7, open columns) following challenge with 0.07–0.2 mg/kg A 68930 or vehicle (V). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over a 60-min period. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ vs. vehicle; $^*P < 0.05$, $^{**}P < 0.01$ vs. wildtype receiving the same dose.

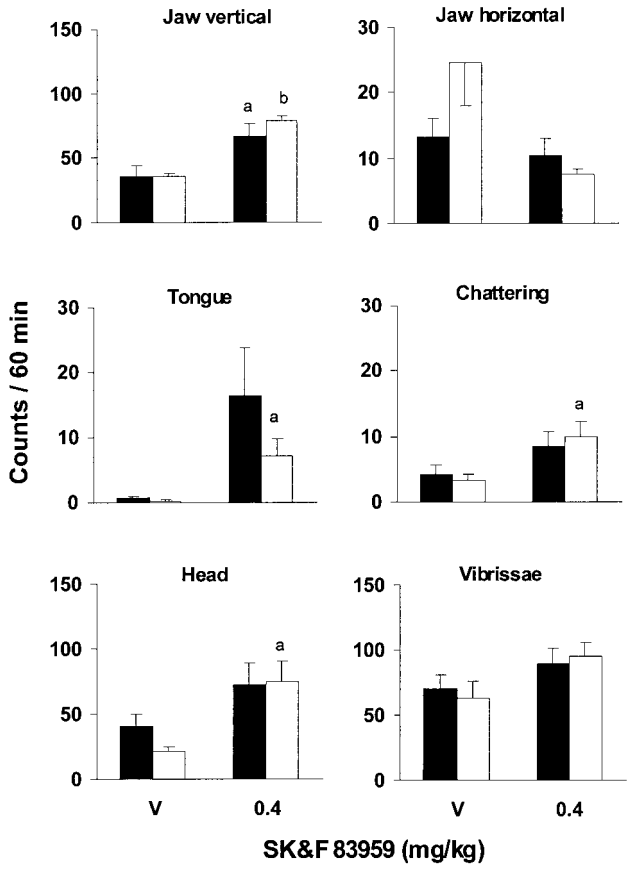

Fig. 6. Phenotype of orofacial movement topographies in wildtypes (n = 5, filled columns) and incipient congenic D_2 mutants (n = 5, open columns) following challenge with 0.4 mg/kg SK&F 83959 or vehicle (V). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over a 60-min period. $^aP < 0.05$, $^bP < 0.01$ vs. vehicle.

General parameters: SK&F 83959 challenge

On examining five male incipient congenic D_2 mutants for orofacial topography in response to SK&F 83959, mean body weight (18 ± 3 g; mean age 221 ± 48 days) was lower (–18%) relative to five male wildtype controls (22 ± 1 g; mean age 340 ± 24 days).

On examining 10 male congenic D_3 mutants for orofacial topography in response to SK&F 83959, mean body weight (27 ± 1 g; mean age 146 ± 9 days) did not differ from 10 male wildtype controls (26 ± 1 g; mean age 144 ± 8 days).

SK&F 83959-induced orofacial topography

D_2 mutants

In wildtypes, SK&F 83959 induced vertical jaw movements, tongue protrusions, and incisor chattering and reduced horizontal jaw movements (Fig. 6), These responses were unaltered in congenic D_2 mutants (overall effects of dose: vertical jaw movements, $F(1,16) = 27.94$, $P < 0.001$; tongue protrusions, $F(1,16) = 16.27$, $P < 0.001$; incisor chattering, $F(1,16) = 9.86$, $P < 0.01$; horizontal jaw movements, $F(1,16) = 5.61$, $P < 0.05$; no overall effects of genotype or dose × genotype interactions). Similarly, SK&F 83959 induced general head and vibrissae movements in a manner that did not differ between the genotypes (overall effects of dose: head movements, $F(1,16) = 15.38$, $P < 0.01$; vibrissae movements, $F(1,16) = 5.15$, $P < 0.05$; no overall effects of genotype or dose × genotype interactions).

D_3 mutants

In wildtypes, SK&F 83959 induced vertical jaw movements, tongue protrusions, and incisor chattering and reduced horizontal jaw movements (Fig. 7). These responses were unaltered in congenic D_3 mutants (overall effects of dose: vertical jaw movements, $F(2,34) = 70.68$, $P < 0.001$; tongue protrusions, $F(2,34) = 5.43$, $P < 0.01$; incisor chattering, $F(2,34) = 7.06$, $P < 0.01$; horizontal jaw movements, $F(2,34) = 8.47$, $P < 0.001$; no overall effects of genotype or dose ×

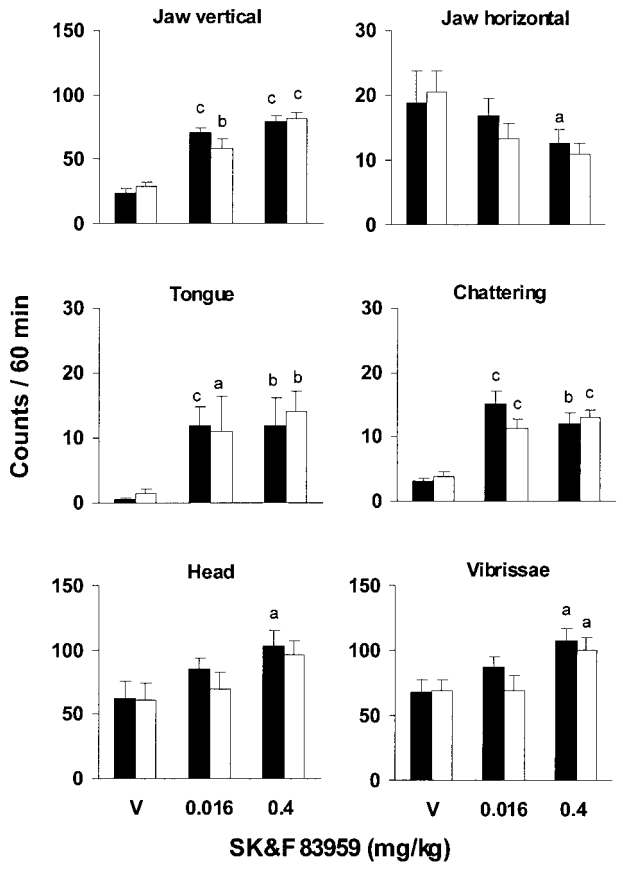

Fig. 7. Phenotype of orofacial movement topographies in wildtypes (n = 6–7, filled columns) and congenic D_3 mutants (n = 6–7, open columns) following challenge with 0.016–0.4 mg/kg SK&F 83959 or vehicle (V). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements over a 60-min period. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ vs. vehicle.

genotype interactions). Similarly, SK&F 83959 induced general head and vibrissae movements in a manner that did not differ between the genotypes (overall effects of dose: head movements, $F(2,34) = 21.65$, $P < 0.001$; vibrissae movements, $F(2,34) = 42.43$, $P < 0.001$; no overall effects of genotype or dose × genotype interactions).

DISCUSSION

These studies seek to clarify the functional roles of individual DA receptor subtypes, specifically here the involvement of members of the D_2-like family in regulating orofacial movements (Rosengarten et al., 1983, 1986; Murray and Waddington, 1989; Collins et al., 1991; Waddington et al., 1998; Niznik et al., 2002). Described here are such phenotypic aspects of mice with incipient congenic D_2 and congenic D_3 receptor knockout.

Methodological issues

A number of methodological refinements have been incorporated into these studies: 1) Because of general concerns over potential effects of knockout on a mixed (hybrid) genetic background, which might influence apparent phenotype independent of the entity deleted (Gerlai, 1996; Crawley et al., 1997; Kelly et al., 1998; Phillips et al., 1999; Waddington et al., 2001, McNamara et al., 2003), incipient congenic D_2 and congenic D_3 mutant lines were established by repeated backcrossing into a single strain. This work, with counterpart studies of orofacial movement topography in a congenic D_{1A} mutant line (Tomiyama et al., 2002), constitutes a first systematic examination of such phenotype in a congenic D_2 or D_3 mutant line. 2) To allow the detailed study of orofacial movements in the mouse, a novel system was utilised (Tomiyama et al., 2001). 3) A physiologically based approach to the resolution and quantification of orofacial movements was adopted, with specification of individual topographies rather than recourse to widely adopted, generic terms such as "vacuous chewing" which composite multiple components that may be regulated differentially (Waddington, 1990; Waddington et al., 1998). This approach allowed resolution in wildtypes of four topographies: vertical jaw movements, horizontal jaw movements, tongue protrusions, and incisor chattering, as well as general movements of the head and vibrissae. Comparisons are made with our recent report on these same parameters, assessed using identical methods by the same investigators, in congenic D_{1A} mutants (Tomiyama et al., 2002).

Spontaneous orofacial topography

Under spontaneous conditions, vertical jaw movements were increased in incipient congenic D_2 mutants in a manner that did not change materially with time, but were unaltered in congenic D_{1A} mutants. Conversely, horizontal jaw movements were unaltered in D_2 mutants but essentially abolished in D_{1A} mutants. Thus, the present approach indicates that apparent oppositional D_1-like:D_2-like interactions in the regulation of composited jaw movements (Waddington et al., 1994) may in fact reflect the independent actions of D_2 receptors to inhibit vertical jaw movements and of D_{1A} receptors to facilitate horizontal jaw movements. These differential regulatory effects may be masked by a composite index of jaw movements and are revealed only on resolving individual topographies of behaviour. There appears to be little material involvement of D_3 receptors in these processes.

Low baseline levels of tongue protrusions and incisor chattering were reduced in D_2 mutants and in D_{1A} mutants in a time-dependent fashion, but were less altered in D_3 mutants. These findings indicate distinct, facilitatory rather than inhibitory roles in their regulation. Among general head and vibrissae movements, only the former were reduced and only in D_2 mutants. Conversely, D_{1A} mutants evidenced an altered time-course of habituation for these movements, which

would have gone unrecognised using composite indices over shorter periods of assessment.

Thus, the present findings extend to the oral domain our recent findings that D_2, and less so D_3, receptors exert essentially motoric effects on exploratory behaviour. While initial studies in D_2 mutants on a mixed genetic background indicated impaired motor function and some Parkinsonian features (Baik et al., 1995; Jung et al., 1999), motor deficits in D_2 mutants were subsequently reported to be much less prominent both on a mixed (Clifford et al., 2000) and on an incipient congenic (Kelly et al., 1998; Clifford et al., 2001) background. Although the present abnormalities in orofacial movement were found in the same incipient congenic D_2 mutants which showed less prominent deficits in motor function (Kelly et al., 1998; Clifford et al., 2001), the relationship of deficits in motor function to abnormalities in orofacial movements is not clear; while striatal D_2 receptors are likely to be involved, the contribution of extrastriatal limbic and possibly cortical mechanisms cannot be discounted. Also, we cannot exclude the possibility of different effects on orofacial movements in D_2 mutants on a mixed background.

Conversely, D_{1A} receptors also influence psychomotor processes that regulate change in behaviour over time as an animal interacts with and habituates to its environment (Clifford et al., 1998, 2001; McNamara et al., 2002, 2003), as noted here in relation to orofacial movement. As for D_2 receptors, while striatal D_{1A} receptors are likely to be involved, extrastriatal limbic and possibly cortical mechanisms may also contribute to these processes.

As with all "knockouts," it cannot be excluded that aspects of phenotype are influenced also by compensatory mechanisms consequent to the developmental absence of the entity deleted (Clifford et al., 1998, 2000, 2001; Kelly et al., 1998; Sibley, 1999; Waddington et al., 2001; Tomiyama et al., 2002; McNamara et al., 2003).

D_2-like agonist-induced orofacial topography

The D_2-like agonist RU 24213 (Euvrard et al., 1980; Clifford et al., 2000; McNamara et al., 2002) reduced vertical and particularly horizontal jaw movements; this effect on horizontal jaw movements was partially reduced in incipient congenic D_2 but not in congenic D_3 mutants. This could suggest, in addition to D_2 mechanisms, some additional involvement of D_4-mediated or non-DAergic effects. These findings are complementary to the D_{1A} receptor exerting a primary, facilitative role in the regulation of horizontal jaw movements under spontaneous conditions and only a minor role under RU 24213 challenge. Low baseline levels of tongue protrusions and incisor chattering, with head and vibrissae movements, were not influenced in any substantive manner by RU 24213, without prominent differences between the genotypes. This indicates some specificity of RU 24213 to influence jaw movements in the horizontal plane. Only in relation to head movements did a low but not a high dose of RU 24213 exert an inhibitory effect, and this effect was attenuated in congenic D_3 mutants. This complements attenuation in congenic D_3 mutants of the effect of a low dose of RU 24213 to reduce only certain topographies of exploratory behaviour and only following a similar period of habituation (McNamara et al., 2002).

D_1-like agonist-induced orofacial topography

A 68930 is a D_1-like agonist of high selectivity and full efficacy to stimulate adenylyl cyclase (DeNinno et al., 1991; Daly and Waddington, 1993; Clifford et al., 2001). That it induced vertical, but not horizontal, jaw movements which were unaltered in incipient congenic D_2 mutants would suggest mediation via D_1-like receptors independent of D_2 receptor modulation. Conversely, A 68930-induced tongue protrusions were reduced in D_2 mutants, suggesting a facilitatory role for D_2 receptors in this D_1-like agonist-induced response. Furthermore, both spontaneous and A 68930-induced incisor chattering were reduced in D_2 mutants, suggesting a facilitatory role for D_2 receptors in this orofacial topography.

SK&F 83959 shows high selectivity for D_1-like over D_2-like receptors at which it acts to inhibit rather than stimulate DA-sensitive adenylyl cyclase (Arnt et al., 1992; Andringa et al., 1999; Gnanalingham et al., 1995) yet it shows all the behavioural characteristics of cyclase-stimulating D_1-like agonists, including induction in rats of "vacuous chewing"/perioral movements (Arnt et al., 1992; Deveney and Waddington, 1995; Adachi et al., 1999; Hasegawa et al., 2001) and in mice of vertical but not horizontal jaw movements together with tongue protrusions and incisor chattering (Tomiyama et al., 2001), in a manner similar to A 68930. Thus, it may act at a putative D_1-like site linked to a transduction system other than / additional to adenylyl cyclase, with phosphoinositide turnover being the most widely entertained candidate (Mahan et al., 1990; Undie and Friedman, 1990; Undie et al., 1994; Waddington et al., 1995, 1998; Panchalingham and Undie, 2001; Niznik et al., 2002). The effects of D_2 receptor ablation on topographical responsivity to SK&F 83959, confined to some attenuation of tongue protrusions, were similar to effects on responsivity to A 68930; conversely, such responsivity was essentially abolished in D_{1A} mutants (Tomiyama et al., 2002).

On the basis of evidence in D_3-null mice on a mixed genetic background (Xu et al., 1997; Jung and Schmauss, 1999; Karasinska et al., 2000), and in other paradigms (Levavi-Sivan et al., 1998; Ridray et al., 1998; Schwartz et al., 1998) it has been suggested that D_3 receptors may participate in and modulate aspects of well-described D_1-like:D_2-like interactions that are fundamental regulators of DAergic function (Wadding-

ton et al., 1994, 2001). Congenic D_3 mutants evidenced no material alteration in the topography of orofacial movements in response to these D_1-like agonists, indicating no substantive role for the D_3 receptor either in their genesis or in their modulation.

Regulation of orofacial movement topography

These findings help to resolve the individual roles of D_2 and D_3 receptors in orofacial topographies mediated through D_2-like receptors, yet some anomalies are apparent. For example, A 68930-induced orofacial topographies were either uninfluenced or diminished in D_2 mutants; however, we have recently reported A 68930-induced "vacuous chewing" to be enhanced in these same incipient congenic D_2 mutants under more naturalistic conditions, in accordance with their regulation by oppositional D_1-like:D_2-like interactions (Waddington et al., 2001; Clifford et al., 2001). It should be emphasised that "vacuous chewing" constitutes a generic term which composites into a single measure a diversity of orofacial movements as assessed under naturalistic circumstances, while the present study involves resolution of specific topographies of orofacial movement assessed under restraint. In particular, it remains difficult to resolve the paradox that naturalistic assessment, while constituting a more physiologically relevant situation, precludes detailed topographical resolution, while restraint allows such topographical resolution but under less physiological circumstances. Such factors may contribute to these differing profiles of effect of D_2 receptor ablation between the two conditions. Thus, an important methodological issue is highlighted (Waddington et al., 2001): profiles of phenotypic effect of gene deletion obtained under one set of conditions may not necessarily generalise to other conditions.

Furthermore, some differences in the effect of D_2 receptor ablation were evident according to whether topographies of orofacial movement were occurring spontaneously or induced by D_2-like vs. D_1-like selective agonists. This would appear to attest the following realities: under spontaneous conditions, all DA receptor subtypes are subjected to endogenous, tonic stimulation by DA; conversely, under challenge with agonists, specific families of DA receptor subtypes are subjected to exogenous, phasic stimulation, on a background of possibly modified endogenous, tonic stimulation by DA. Such physiological differences may contribute to differing profiles of effect of D_2 receptor ablation between the two conditions.

In summary, D_2 receptors exert differential regulation of individual orofacial movement topographies in a manner that is distinct from, and sometimes opposite to, that of their D_{1A} counterparts. Conversely, D_3 receptors exert only minor regulation of such movements.

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