UNIVERSITY OF MEDICINE AND HEALTH SCIENCES

Royal College of Surgeons in Ireland

repository@rcsi.com

Disruption of orofacial movement topographies in congenic mutants with dopamine D5 but not D4 receptor or DARPP-32 transduction 'knockout'

AUTHOR(S)

Katsunori Tomiyama, Yasuyuki Makihara, Hiroshi Yamamoto, Gerard J. O'Sullivan, Rachel E. Nally, Orna Tighe, Anthony Kinsella, Allen A. Fienberg, David K. Grandy, David R. Sibley, David T. Croke, Noriaki Koshikawa, John L. Waddington

CITATION

Tomiyama, Katsunori; Makihara, Yasuyuki; Yamamoto, Hiroshi; O'Sullivan, Gerard J.; Nally, Rachel E.; Tighe, Orna; et al. (2006): Disruption of orofacial movement topographies in congenic mutants with dopamine D5 but not D4 receptor or DARPP-32 transduction 'knockout'. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.10783832.v1

HANDLE

10779/rcsi.10783832.v1

LICENCE

CC BY-NC-ND 4.0

This work is made available under the above open licence by RCSI and has been printed from https://repository.rcsi.com. For more information please contact repository@rcsi.com

URL

https://repository.rcsi.com/articles/journal_contribution/Disruption_of_orofacial_movement_topographies_in_congenic_mutants_with_dopamine_D5_but_not_D4_receptor_or_DARPP-32_transduction_knockout_/10783832/1

Disruption of orofacial movement topographies in congenic mutants with dopamine D_5 but not D_4 receptor or DARPP-32 transduction 'knockout'

Katsunori Tomiyama[a], Yasuyuki Makihara[b], Hiroshi Yamamoto[c], Gerard O'Sullivan[e], Rachel E. Nally[e], Orna Tighe[f], Anthony Kinsella[g], Allen A. Fienberg[h], David K. Grandy[i], David R. Sibley[j], David T. Croke[f], Noriaki Koshikawa[a,d], John L. Waddington[e]*

[a]Nihon University Advanced Research Institute for the Sciences and Humanities, Tokyo 102, Japan

[b]Departments of Pharmacology and [c]Oral and Maxillofacial Surgery, and [d]Dental Research Centre, Nihon University School of Dentistry, Tokyo 101, Japan

[e]Departments of Clinical Pharmacology and [f]Biochemistry, and Research Institute, Royal College of Surgeons in Ireland, Dublin 2, Ireland

[g]School of Mathematics, Dublin Institute of Technology, Dublin 8, Ireland

[h]Laboratory of Molecular & Cellular Neuroscience, The Rockefeller University, New York, NY 10021, USA

[i]Department of Physiology and Pharmacology, Oregon Health & Science University, Portland, OR 97239, USA

[j]Molecular Neuropharmacology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA

*Address for correspondence:

Dr. John L. Waddington,

Department of Clinical Pharmacology,

Royal College of Surgeons in Ireland,

St Stephen's Green,

Dublin 2,

Ireland.

Tel: +353-1-402 2245

Fax: +353-1-402 2453

Email: jwadding@rcsi.ie (J.L. Waddington)

Abstract

The role of D_1-like [D_1, D_5] and D_2-like [D_2, D_3, D_4] dopamine receptors and dopamine transduction via DARPP-32 in topographies of orofacial movement was assessed in restrained mice with congenic D_4 vs D_5 receptor vs DARPP-32 'knockout'. D_4 and DARPP-32 mutants evidenced no material phenotype; also, there were no alterations in topographical responsivity to either the selective D_2-like agonist RU 24213 or the selective D_1-like agonist SK&F 83959. In contrast, D_5 mutants evidenced an increase in spontaneous vertical jaw movements, which habituated more slowly than in wildtypes, and a decrease in horizontal jaw movements; topographical responsivity to SK&F 83959 and RU 24213 was unaltered. D_5 receptors regulate distinct topographies of vertical and horizontal jaw movement in an opposite manner. In assuming that the well-recognised role of the D_1-like family in regulating orofacial movements involves primarily D_1 receptors, a role for their D_5 counterparts may have been overlooked.

Key words: Orofacial movements; Dopamine receptors; D_4 'knockout'; D_5 'knockout'; DARPP-32 'knockout'; topographical assessment

1. Introduction

Regulation of orofacial movements constitutes a fundamental process given their criticality for consummatory behaviour, defensive and attack behaviours, self-care, vocalisation and, in higher mammals, verbal as well as non-verbal communication. While it is well recognized that the D_1-like family of dopamine (DA) receptors exerts an important role in the regulation of orofacial movements (Rosengarten et al., 1983, 1986; Murray and Waddington, 1989; Collins et al., 1991; Waddington et al., 1995; Niznik et al., 2002), parcellating this role between individual family members [D_1 (also known as D_{1A}), D_5 (also known as D_{1B})] has proved difficult. Specifically, in the absence of agents that can discriminate materially between D_1 and D_5 receptors, it has been assumed that the D_1 receptor exerts a prepotent, if not exclusive, role on the basis of its high density localisation in brain regions such as the striatum, which are known to exert a fundamental role in the regulation of orofacial movements; conversely, the D_5 receptor has a low density localisation in primarily cortical and limbic regions (Khan et al., 2000; Di Chiara, 2002).

The D_2-like family plays a lesser role in the regulation of orofacial movements, both independently and especially via D_1-like:D_2-like interactions (Waddington et al., 1994, 1995). Similarly, in the absence of agents that can discriminate materially between D_2, D_3 and D_4 receptors, it has been assumed that the D_2 receptor exerts a prepotent, if not

exclusive, role on the basis of its high density localisation in the striatum; conversely, D_3 and particularly D_4 receptors have a lower density striatal and primarily corticolimbic localisation (Tarazi and Baldessarini, 1999; Di Chiara, 2002).

The phosphoprotein DARPP-32 [dopamine- and adenosine 3′,5′-monophosphate-regulated phosphoprotein of 32 kilodaltons] is a critical component in the DA signaling cascade following activation both of D_1-like and of D_2-like receptors, particularly in relation to D_1 and D_2 receptors but possibly extending to other family members (Greengard et al., 1999; Greengard, 2001). However, any role in the regulation of orofacial movement topography is unknown.

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). However, it is only recently that their potential to clarify the roles of individual DA receptor subtypes in regulating orofacial movements has been realised. Indeed, systematic assessment of such movements is only now being undertaken 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).

We have 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; Makihara et al., 2004). This has been applied to describe the phenotype of orofacial movements and topographical responses to D_1-like and D_2-like agonists in mice with congenic D_1, D_2 and D_3 receptor 'knockout' (Tomiyama et al., 2002, 2004). We now describe the application of this technique to characterise topographically, in a complementary, comparative manner, the phenotype of orofacial movements, and responses to the D_1-like agonist SK&F 83959 and the D_2-like agonist RU 24213, in mice with congenic D_4 vs D_5 receptor vs DARPP-32 'knockout'.

2. Experimental procedures

2.1. Animals

The original F2 hybrid strain [129/Ola × C57BL/6J] containing the mutated D_4 receptor allele was generated as reported previously (Rubinstein et al., 1997); animals from an essentially congenic line of D_4 'knockouts' [10 back-crosses into C57BL/6J]

were shipped to Dublin, where PCR analysis was used to identify congenic, homozygous mutants [$D_4^{-/-}$] and wildtypes [$D_4^{+/+}$] among the progeny of homozygous intermatings. The original F2 hybrid strain [129/SvJ1 × C57BL/6] containing the mutated D_5 receptor allele was generated as reported previously (Hollon et al., 2002); animals from an essentially congenic line of D_5 knockouts [7 back-crosses into C57BL/6] were shipped to Dublin, where PCR analysis was used to identify congenic, homozygous mutants [$D_5^{-/-}$] and wildtypes [$D_5^{+/+}$] among the progeny of heterozygous [$D_5^{+/-}$] intermatings (O'Sullivan et al., 2005). The original F2 hybrid strain [129/Ola × C57BL/6] containing the mutated DARPP-32 allele was generated as reported previously (Fienberg et al., 1998); animals from an essentially congenic line of DARPP-32 knockouts [10 back-crosses into C57BL/6] were shipped to Dublin, where PCR analysis was used to identify congenic, homozygous mutants [DARPP-$32^{-/-}$] and wildtypes [DARPP-$32^{+/+}$] among the progeny of homozygous intermatings (Nally et al., 2003).

Following establishment of these breeding colonies in Dublin, experimental animals were shipped to Tokyo. There, they were maintained at 23 ± 1 ^{o}C and 55 ± 5% humidity on a 12 h /12 h (07.00 on; 19.00 off) light/dark schedule, with food and water available *ad libitum*. While approximately 75% of animals remained group-housed, approximately 25% of animals engaged in injurous fighting and were subsequently housed singly to conserve adequate experimental numbers; the necessity for such

housing occurred randomly and did not differ between the genotypes. Thereafter, they were maintained undisturbed for an adaptation period of at least two weeks before experiments were commenced. Young adult mice from litters of the same generational age were used in behavioural assessments, which took place between 08.30 and 11.30 to reduce any effects of circadian variation in orofacial movements. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland and the Animal Experimentation Committee of Nihon University School of Dentistry, and were conducted under license from the Department of Health and Children in accordance with European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

2.2. 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 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 minimize visual disruption and partially reduce auditory interference. 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.

2.3. 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., 2000, 2001; Ross et al., 2000; McNamara et al., 2002, 2003; O'Sullivan et al., 2005), was applied similarly to resolve the topography of orofacial movement (Tomiyama et al., 2001, 2002): each of five mice was observed sequentially for 5 s periods at 25 s 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 s periods. For assessment of orofacial movement topography and its habituation profile without drug challenge, 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 drug 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 60 min; mice were used on two occasions only, separated by a drug-free interval of at least one week, with random allocation to one of the various treatments in each instance. All observations were made by an observer who was unaware of genotype and treatment for each animal.

2.4. 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 selective D_1-like agonists SK&F 83959 [3-methyl-6-chloro-7,8-dihydroxy-1-[3-methyl-phenyl]-2,3,4,5-tetrahydro-1*H*-3-benzaz epine; RBI/SRI/NIMH Chemical Synthesis Program, USA] and A 68930 [*cis*-(±)-1-(aminomethyl)-3,4-dihydro-3-phenyl-1*H*-2-benzopyran-5,6-diol hydrochloride; Tocris Cookson Ltd., UK] were dissolved in distilled water. Drugs or vehicle were injected subcutaneously into the flank in a volume of 2 ml/kg.

2.5. Data analysis

For determination of habituation profiles of orofacial movement topographies under restraint in the absence of drug treatment, 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 administration. Data were expressed as means ± S.E.M. and analysed using repeated-measures analysis of variance (ANOVA) after square-root transformation in the absence of appropriate non-parametric techniques; univariate comparisons were made where overall effects on ANOVA were significant, using Student's *t*-test or Mann-Whitney *U*-test after Kruskal-Wallis analysis as appropriate (McNamara et al., 2002, 2003; Ross et al., 2000; Tomiyama et al., 2001, 2002, 2004; Makihara et al., 2004; Nally et al., 2003,

2004; O'Sullivan et al., 2004, 2005).

3. Results

3.1. General parameters

On examining 19 male congenic D_4 mutants for orofacial topography under restraint, mean body weight [29 ± 1 g; mean age 178 ± 5 days] did not differ from 19 wildtype controls [29 ± 1 g; mean age 174 ± 7 days]; No gross motor phenotype was apparent when animals were observed qualitatively for posture, reactivity to handling and general activity (see also Rubinstein et al., 1997).

On examining 20 male congenic D_5 mutants for orofacial topography under restraint, mean body weight [29 ± 1 g; mean age 180 ± 4 days] did not differ from 20 male wildtype controls [29 ± 1 g; mean age 180 ± 2 days]. No gross motor phenotype was apparent when animals were observed similarly (see also Holmes et al., 2001; Hollon et al., 2002; O'Sullivan et al., 2005).

On examining 20 male congenic DARPP-32 mutants for orofacial topography under restraint, mean age [152 ± 5 days; mean body weight 27 ± 1 g] was slightly older than that of 18 male wildtype controls [136 ± 2 days, $P < 0.01$; mean body weight 27 ± 1 g]; the results of analyses reported below were unaltered on including age and weight

as covariates. No gross motor phenotype was apparent when animals were observed similarly (see also Fienberg et al., 1998; Nally et al., 2003, 2004).

3.2. Topography of orofacial movements over habituation

In wildtypes, vertical jaw movements, with movements of the head and vibrissae, were prominent initially but declined subsequently, while horizontal jaw movements increased in prominence over the habituation period; tongue protrusion and incisor chattering were present at modest levels throughout habituation (Fig. 1).

In congenic D_4 mutants, this topography of orofacial movements was essentially unaltered [no effects of genotype or genotype × time interactions] (data not shown).

In congenic D_5 mutants (Fig. 1), levels of vertical jaw movements were increased, and they habituated more slowly than in wildtypes [overall effect of genotype, $F(1, 38) = 5.64$, $P < 0.05$; genotype × time interaction, $F(3, 114) = 4.54$, $P < 0.005$]. Conversely, levels of horizontal jaw movements [overall effect of genotype, $F(1, 38) = 12.54$, $P < 0.005$; no genotype × time interaction] and of vibrissae movements [overall effect of genotype, $F(1, 38) = 5.16$, $P < 0.05$; no genotype × time interaction] were decreased. Other topographies of orofacial movement were essentially unaltered.

In congenic DARPP-32 mutants (Fig. 2), tongue protrusions were decreased [overall effect of genotype, $F(1, 36) = 12.81$, $P < 0.01$; no genotype × time interaction].

Other topographies of orofacial movement were essentially unaltered.

3.3. Topography of drug-induced orofacial movements

In wildtypes, 0.016-0.4 mg/kg SK&F 83959 readily induced vertical but not horizontal jaw movements, with tongue protrusions and incisor chattering, together with movements of the head and vibrissae (Fig. 3); conversely, 0.1-1.0 mg/kg RU 24213 reduced horizontal but not vertical jaw movements, with little effect on other topographies of orofacial movement (data not shown). These profiles of agonist responses were similar to those described previously in C57BL/6J mice (Tomiyama et al., 2001; Makihara et al., 2004).

In congenic D_4 mutants, the effects of SK&F 83959 to induce vertical jaw movements, tongue protrusions, incisor chattering and head movements were unaltered; there was slight variation in the induction of vibrissae movements between D_4 mutants and wildtypes with increasing dose of SK&F 83959 [genotype × dose interaction, $F(2, 30) = 3.81$, $P < 0.05$; no other genotype × dose interactions] (Fig. 3). The effect of RU 24213 to reduce horizontal jaw movements was unaltered (data not shown).

In congenic D_5 mutants, these effects of SK&F 83959 and RU 24213 were essentially unaltered [no overall effects of genotype or genotype × dose interactions] (data not shown).

In congenic DARPP-32 mutants, these effects of SK&F 83959 and RU 24213 were essentially unaltered [no overall effects of genotype or genotype × dose interactions] (data not shown). In DARPP-32 mutants, studies with an additional D_1-like agonist, A 68930, were performed [see Discussion]; this agent induced vertical but not horizontal jaw movements, with tongue protrusions and incisor chattering, in a manner indistinguishable from SK&F 83959, as described previously (Tomiyama et al., 2001). These responses were unaltered in DARPP-32 mutants challenged with 0.068-0.2 mg/kg A 68930; levels of horizontal jaw movements were increased in DARPP-32 mutants in a manner unrelated to dose of A 68930 [overall effect of genotype, $F(1, 32) = 7.97$, $P < 0.01$; no genotype × dose interaction] (Fig. 4).

4. Discussion

Given the criticality of orofacial movements for a variety of fundamental mammalian behaviours [see **1. Introduction**], these studies apply mutant mice to investigate the roles of D_4 and D_5 members of, respectively, the D_2-like and D_1-like families of DA receptor subtypes and the DA transduction mediator DARPP-32 in their regulation. A number of methodological refinements have been incorporated into these studies: each 'knockout' is on a congenic as opposed to a mixed [hybrid] genetic background; a novel system is applied to allow the resolution and

quantification of individual orofacial movement topographies; and comparisons are made with our recent reports on these same parameters, assessed using identical methods by the same investigators, in congenic D_1, D_2 and D_3 mutants, as described previously in detail (Tomiyama et al., 2001, 2002, 2004). It should be emphasized that the present findings were conducted in male mutants. Therefore, the extent to which these findings generalize to females is unclear until systematic comparisons between the genders are conducted.

Also, it was necessary to house approximately 25% of animals singly [see **2. Experimental procedures**]. Though isolation from weaning can effect dopamine receptor function (Gariepy et al., 1998), the present necessity for such housing occurred randomly at several months of age and did not differ between the genotypes; thus, though it may influence overall levels of orofacial movement, it is unlikely to account for phenotypic differences between genotypes. Furthermore, the present study involves resolution of orofacial movement under the stress of restraint. Though restraint is necessary to allow detailed topographical resolution in the mouse that is not possible under naturalistic conditions (Tomiyama et al., 2001), it cannot be excluded that some of the present effects are influenced by an interaction between genetic mutation and stress of restraint.

Under these conditions, congenic D_4 mutants evidenced no phenotypic effects in terms of orofacial movement topography under restraint. In relation to the D_2-like

family, this would complement our findings in D_2 and D_3 mutants that it is the D_2 receptor that exerts some regulatory role to inhibit vertical jaw movements, with a limited role to facilitate tongue protrusions and incisor chattering, while D_3 receptors exert only minor regulatory effects (Tomiyama et al., 2002, 2004). This profile may be in keeping with the known anatomical and physiological localisation of these receptors: D_2 receptors have a dense localisation within the striatum, a region known to be critical for the regulation of orofacial movements; D_3 receptors have a lower density localisation within circumscribed striatal regions and corticolimbic structures; D_4 receptors have a low density striatal and a particular corticolimbic localisation (Tarazi and Baldessarini, 1999; Di Chiara, 2002; Rivera et al., 2002a). However, 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., 2000, 2001; Kelly et al., 1998; Sibley, 1999; Waddington et al., 2001; McNamara et al., 2002, 2003; Tomiyama et al., 2002, 2004).

In contrast, congenic D_5 mutants under restraint evidenced an increase in vertical jaw movements, with delayed habituation, and a decrease in horizontal jaw movements and in movements of the vibrissae. In relation to the D_1-like family, we have reported previously that D_1 mutants evidence markedly reduced horizontal jaw movements, delayed habituation in vertical jaw movements, with reduction in tongue protrusions and incisor chattering (Tomiyama et al., 2002). This profile would be less in keeping

with the known anatomical and physiological localisation of these receptors: while D_1 receptors have a dense localisation within the striatum and in certain cortical regions, D_5 receptors have a low density striatal and a particular corticolimbic localisation (Khan et al., 2000; Di Chiara, 2002); yet D_5 mutants exhibit phenotypic effects in terms of orofacial movement topography. Furthermore, the present differential regulatory effects of D_5 vs D_1 receptors would have been obscured by a composite index of jaw movements, such as widely-studied 'vacuous chewing', applied over what is commonly a limited time-frame: these differences are apparent only on resolving individual topographies of orofacial movement as they change over the prolonged process of habituation.

Given the status of DARPP-32 as a critical mediator in the DA signaling cascade particularly through D_1 and D_2 receptors (Greengard et al., 1999; Greengard, 2001), the absence of any substantive orofacial movement phenotype in congenic DARPP-32 mutants other than a decrease in tongue protrusions might be unexpected given the clear phenotypic effects in D_1 and D_2 mutants. However, these findings are consistent with our recent report (Nally et al., 2003) that DARPP-32 mutants show only a subtle phenotype in an ethologically based paradigm that resolves all components of behaviour in the mouse repertoire over the course of initial exploration of and subsequent habituation to the environment. Developmental absence of DARPP-32 in these mutants makes it possible that compensatory processes will be recruited to sustain functions

usually mediated by DARPP-32 under normal conditions (Waddington et al., 2001; Nally et al., 2003, 2004).

Neither D_4 nor D_5 mutants evidenced material alterations in topographical responsivity to the D_1-like agonist SK&F 83959 (Deveney et al., 1995; Niznik et al., 2002), which evidences similar affinities for D_1 and D_5 receptors over their D_2-like counterparts (O'Sullivan et al., 2004), or to the D_2-like agonist RU 24213 (Clifford et al., 2001; McNamara et al., 2002), which evidences similar affinities for D_2, D_3 and D_4 receptors over their D_1-like counterparts (Roth, personal communication; O'Sullivan et al., in preparation). For D_4 mutants this may reflect the limited involvement of D_4 receptors in such processes, even though a presence in the striatum is increasingly recognized (Rivera et al., 2002a), while for D_5 mutants this may reflect in part a controversy as to the functional roles of putative D_1-like receptors that are linked to a transduction mechanism other than/additional to adenylyl cyclase, and where the status of D_5 receptors remains particularly unclear (Niznik et al., 2002); in D_1 mutants, topographical orofacial movement responses to SK&F 83959 are attenuated (Tomiyama et al., 2002).

Similarly, DARPP-32 mutants evidenced unaltered topographical responsivity to SK&F 83959 and RU 24213. The role of DARPP-32 in DA receptor signal transduction involves particularly D_1-like receptors linked to adenylyl cyclase (Greengard et al., 1999; Greengard, 2001), while SK&F 83959 is a D_1-like agonist which has actions at

putative D_1-like receptors linked to an alternative transduction system, perhaps phosphoinositide hydrolysis (Panchalingam and Undie, 2001; Jin et al., 2003). Therefore the isochroman A 68930, a full efficacy agonist at adenylyl cyclase-coupled D_1-like receptors (DeNinno et al., 1990; Niznik et al., 2002), was also studied in DARPP-32 mutants. Topographical responses to A 68930 were unaltered, with an effect of genotype in the absence of any dose × genotype interaction indicating an overall increase in horizontal jaw movements in DARPP-32 mutants that was unrelated to treatment; this echoes a similar increase in general topographies of behaviour in DARPP-32 mutants that we have reported previously using an ethologically based paradigm, and which may reflect a subtle action of the stress of the injection procedure to reveal phenotypic effects not present under other conditions (Nally et al., 2003, 2004). Irrespective of these considerations, developmental absence of DARPP-32 is not associated with material alteration in topographical orofacial movement responses to D_1-like and D_2-like agonists; thus, the operation of compensatory processes may be relevant.

In conclusion, the present data on orofacial movement topographies under restraint may suggest some involvement of cortical D_5 mechanisms, as D_5 receptors appear present in cortical regions that show some overlap with those regulating orofacial movement, at least in rats and non-human primates, and cortical regions can influence striatal DAergic function via well-described corticostriatal pathways (Zhang and

Sasamoto, 1990; Takada et al., 1998; Khan et al., 2000; Cools et al., 2002); our own work in rats indicates a role for cortical D_1-like receptors in the regulation of orofacial movements, using pharmacological tools that cannot distinguish between D_1 and D_5 receptors (Adachi et al., 2003). Alternatively, recent evidence suggests that the presence and physiological role of D_5 receptors in the striatum and related basal ganglia regions may have been underestimated (Rivera et al., 2002b; Baufreton et al., 2003). Though the site(s) mediating these effects remain to be specified, the present data indicate an unexpected role for D_5 receptors in the regulation of orofacial movements which complements our recent findings in relation to ethologically based assessments of behavioural repertoire (O'Sullivan et al., 2005). Similar to the more marked phenotype in D_1 mutants (Tomiyama et al., 2002), the present findings in D_5 mutants relate to specific orofacial movement topographies and how these change over habituation. This suggests a role for D_5 as well as for D_1 receptors in the processes by which behavioural change is 'sculpted' over time as an organism interacts with its environment.

Acknowledgements

These studies were supported by: a Nihon University research grant, research grants from the Sato Fund and the Dental Research Centre, the Promotion and Mutual Aid Corporation for Private Schools of Japan, a grant for promotion of multidisciplinary

research projects and grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, Japan; DA 12062, DA 10044, MH 39327, MH 40899 and the Peter Jay Sharp Foundation, USA; Science Foundation Ireland, Enterprise Ireland and the Stanley Medical Research Institute in the Research Institute of the Royal College of Surgeons in Ireland under the Higher Education Authority's Programme for Research in Third Level Institutions, Ireland. We thank Hoechst-Marion-Roussel for RU 24213. SK&F 83959 was provided initially by RBI and subsequently by SRI as part of the Chemical Synthesis Program of the National Institute of Mental Health.

References

Adachi, K., Hasegawa, M., Fujita, S., Lee, J., Cools, A.R., Waddington, J.L., Koshikawa, N., 2003. Prefrontal, accumbal [shell] and ventral striatal mechanisms in jaw movements and non-cyclase-coupled dopamine D_1-like receptors. Eur. J. Pharmacol. 473, 47-54.

Baufreton, J., Garret, M., Rivera, A., de la Calle, A., Gonon, F., Dufy, B., Bioulac, B., Taupignon, A., 2003. D5 (not D1) dopamine receptors potentiate burst-firing in neurons of the subthalamic nucleus by modulating an L-type calcium conductance. J. Neurosci. 23, 816-825.

Clifford, J.J., Usiello, A., Vallone, D., Kinsella, A., Borrelli, E., Waddington, J.L., 2000. Topographical evaluation of behavioural phenotype in a line of mice with targeted gene deletion of the D_2 dopamine receptor. Neuropharmacology 39, 382-390.

Clifford, J.J., Kinsella, A., Tighe, O., Rubinstein, M., Grandy, D.K., Low, M., Croke, D.T., Waddington, J.L., 2001. Comparative, topographically-based evaluation of behavioural phenotype and specification of D_1-like:D_2-like interactions in a line of incipient congenic mice with D_2 dopamine receptor 'knockout'. Neuropsychopharmacology 25, 527-536.

Collins, P., Broekkamp, C.L.E., Jenner, P., Marsden, C.D., 1991. Drugs acting at D-1

and D-2 receptors induce identical purposeless chewing in rats which can be differentiated by cholinergic manipulation. Psychopharmacology 103, 503-512.

Cools, A.R., Lubbers, L., van Oosten, R.V., Andringa, G., 2002. SKF 83959 is an antagonist of dopamine D1-like receptors in the prefrontal cortex and nucleus accumbens: a key to its antiparkinsonian effect in animals? Neuropharmacology 42, 237-245.

DeNinno, M.P., Schoenleber, R., Asin, K.E., MacKenzie, R., Kebabian, J.W., 1990. (1R,3S)-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-3-phenyl-1H-2-benzopyran : a potent and selective D1 agonist. J. Med. Chem. 33, 2948-2950.

Deveney, A.M., Waddington, J.L., 1995. Pharmacological characterization of behavioural responses to SK&F 83959 in relation to 'D1-like' dopamine receptors not linked to adenylyl cyclase. Br. J. Pharmacol. 116, 2120-2126.

Di Chiara, G., 2002. Handbook of Experimental Pharmacology: Dopamine in the CNS 1. Heidelberg, Springer.

Fienberg, A.A., Hiroi, N., Mermelstein, P.G., Song, W., Snyder, G.L., Nishi, A., Cheramy, A., O'Callaghan, J.P., Miller, D.B., Cole, D.G., Corbett, R., Haile, C.N., Cooper, D.C., Onn, S.P., Grace, A.A., Ouimet, C.C., White, F.J., Hyman, S.E., Surmeier, D.J., Girault, J., Nestler, E.J., Greengard, P., 1998. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science 281, 838-842.

Gariepy, J.-L., Gendreau, P.L., Cairns, R.B., Lewis, M.H., 1998. D1 dopamine receptors and the reversal of isolation-induced behaviors in mice. Behav. Brain Res. 95, 103-111.

Greengard, P., Allen, P.B., Nairn, A.C., 1999. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron 23, 435-447.

Greengard, P., 2001. The neurobiology of slow synaptic transmission. Science 294, 1024-1030.

Hollon, T.R., Bek, M.J., Lachowicz, J.E., Ariano, M.A., Mezey, E., Ramachandran, R., Wersinger, S.R., Soares-da-Silva, P., Liu, Z.F., Grinberg, A., Drago, J., Young, W.S. 3rd, Westphal, H., Jose, P.A., Sibley, D.R., 2002. Mice lacking D5 dopamine receptors have increased sympathetic tone and are hypertensive. J. Neurosci. 22, 10801-10810.

Holmes, A., Hollon, T.R., Gleason, T.C., Liu, Z., Dreiling, J., Sibley, D.R., Crawley, J.N., 2001. Behavioral characterization of dopamine D5 receptor null mutant mice. Behav. Neurosci. 115, 1129-1144.

Jin, L.Q., Goswami, S., Cai, G., Zhen, X., Friedman, E., 2003. SKF83959 selectively regulates phosphatidylinositol-linked D1 dopamine receptors in rat brain. J. Neurochem. 85, 378-386.

Kelly, M.A., Rubinstein, M., Phillips, T.J., Lessov, C.N., Burkhart-Kasch, S., Zhang, G., Bunzow, J.R., Fang, Y., Gerhardt, G.A., Grandy, D.K., Low, M.J., 1998.

Locomotor activity in D2 dopamine receptor-deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J. Neurosci. 18, 3470-3479.

Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., de la Calle, A., 2000. Dopamine D5 receptors of rat and human brain. Neuroscience 100, 689-699.

Makihara, Y., Yamamoto, H., Inove, J., Tomiyama, K., Koshikawa, N., Waddington, J.L., 2004. Topographical effects of D_1-like dopamine receptor agonists on orofacial movements in mice and their differential regulation via oppositional vs synergistic D_1-like:D_2-like interactions: cautionary observation on SK&F 82958 as an anomalous agent. J. Psychopharmacol. 18, 485-496.

McNamara, F.N., Clifford, J.J., Tighe, O., Kinsella, A., Drago, J., Fuchs, S., Croke, D.T., Waddington, J.L., 2002. Phenotypic, ethologically-based resolution of spontaneous and D_2-like vs D_1-like agonist-induced behavioural topography in mice with congenic D_3 dopamine receptor 'knockout'. Synapse 46, 19-31.

McNamara, F.N., Clifford, J.J., Tighe, O., Kinsella, A., Drago, J., Croke, D.T., Waddington, J.L., 2003. Congenic D_{1A} dopamine receptor mutants: ethologically-based resolution of behavioural topography indicates genetic background as a determinant of knockout phenotype. Neuropsychopharmacology 28, 86-99.

Murray, A.M., Waddington, J.L., 1989. The induction of grooming and vacuous chewing by a series of selective D-1 dopamine receptor agonists: two directions of D-1:D-2 interaction. Eur. J. Pharmacol. 160, 377-384.

Nally, R.E., McNamara, F.N., Clifford, J.J., Kinsella, A., Tighe, O., Croke, D.T., Fienberg, A.A., Greengard, P., Waddington, J.L., 2003. Topographical assessment of ethological and dopamine receptor agonist-induced behavioral phenotype in mutants with congenic DARPP-32 'knockout'. Neuropsychopharmacology 28, 2055-2063.

Nally, R.E., Kinsella, A., Tighe, O., Croke, D.T., Fienberg, A.A., Greengard, P., Waddington, J.L., 2004. Ethologically based resolution of D2-like dopamine receptor agonist-versus antagonist-induced behavioral topography in dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32 kDa "knockout" mutants congenic on the C57BL/6 genetic background. J. Pharmacol. Exp. Ther. 310, 1281-1287.

Niznik, H.B., Sugamori, K.S., Clifford, J.J., Waddington, J.L., 2002. D_1-like dopamine receptors: molecular biology and pharmacology. In: Di Chiara, G. (Ed.), Handbook of Experimental Pharmacology: Dopamine in the CNS 1. Heidelberg, Springer, pp. 122-158.

O'Sullivan, G.J., Roth, B.L., Kinsella, A., Waddington, J.L., 2004. SK&F 83822 distinguishes adenylyl cyclase from phospholipase C-coupled dopamine D1-like receptors: behavioural topography. Eur. J. Pharmacol. 486, 273-280.

O'Sullivan, G.J., Kinsella, A., Sibley, D.R., Tighe, O., Croke, D.T., Waddington, J.L., 2005. Ethological resolution of behavioural topography and D_1-like vs D_2-like

agonist responses in congenic D_5 dopamine receptor mutants: identification of D_5:D_2-like interactions . Synapse 55, 201-211.

Panchalingam, S., Undie, A,S., 2001. SKF83959 exhibits biochemical agonism by stimulating [^{35}S]GTP gamma S binding and phosphoinositide hydrolysis in rat and monkey brain. Neuropharmacology 40, 826-837.

Rivera, A., Cuellar, B., Giron, F.J., Grandy, D.K., de la Calle, A., Moratalla, R., 2002a. Dopamine D4 receptors are heterogeneously distributed in the striosomes/matrix compartments of the striatum. J. Neurochem. 80, 219-229.

Rivera, A., Alberti, I., Martin, A.B., Narvaez, J.A., de la Calle, A., Moratalla, R., 2002b. Molecular phenotype of rat striatal neurons expressing the dopamine D5 receptor subtype. Eur. J. Neurosci. 16, 2049-2058.

Rosengarten, H., Schweitzer, J.W., Friedhoff, A.J., 1983. Induction of oral dyskinesia in naïve rats by D-1 stimulation. Life Sci. 33, 2471-2482.

Rosengarten, H., Schweitzer, J.W., Friedhoff, A.J., 1986. Selective dopamine D-2 receptor reduction enhances a D-1 mediated oral dyskinesia in rats. Life Sci. 39, 29-35.

Ross, S.A., Wong, J.Y., Clifford, J.J., Kinsella, A., Massalas, J.S., Horne, M.K., Scheffer, I.E., Kola, I., Waddington, J.L., Berkovic, S.F., Drago, J., 2000 Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. J. Neurosci. 20, 6431-6441.

Rubinstein, M., Phillips, T.J., Bunzow, J.R., Falzone, T.L., Dziewczapolski, G., Zhang, G., Fang, Y., Larson, J.L., McDougall, J.A., Chester, J.A., Saez C., Pugsley, T.A., Gershanik, O., Low, M.J., Grandy, D.K., 1997. Mice lacking dopamine D_4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 90, 991-1001.

Sibley, D.R., 1999. New insight into dopaminergic receptor function using antisense and genetically altered animals. Annu. Rev. Pharmacol. Toxicol. 39, 313-341.

Takada, M., Tokuno, H., Nambu, A., Inase, M., 1998. Corticostriatal projections from the somatic motor areas of the frontal cortex in the macaque monkey: segregation versus overlap of input zones from the primary motor cortex, the supplementary motor area, and the premotor cortex. Exp. Brain Res. 120, 114-128.

Tarazi, F.I., Baldessarini, R.J., 1999. Brain dopamine D_4 receptors: basic and clinical status. Int. J. Neuropsychopharmacol. 2, 41-58.

Tomiyama, K., McNamara, F.N., Clifford, J.J., Kinsella, A., Koshikawa, N., Waddington, J.L., 2001. Topographical assessment and pharmacological characterization of orofacial movements in mice: dopamine D_1-like vs. D_2-like receptor regulation. Eur. J. Pharmacol. 418, 47-54.

Tomiyama, K., McNamara, F.N., Clifford, J.J., Kinsella, A., Drago, J., Tighe, O., Croke, D.T., Koshikawa, N., Waddington, J.L., 2002. Phenotypic resolution of spontaneous and D_1-like agonist-induced orofacial movement topographies in

congenic dopamine D_{1A} receptor 'knockout' mice. Neuropharmacology 42, 644-652.

Tomiyama, K., McNamara, F.N., Clifford, J.J., Kinsella, A., Drago, J., Fuchs, S., Grandy, D.K., Low, M.J., Rubinstein, M., Tighe, O., Croke, D.T., Koshikawa, N., Waddington, J.L., 2004. 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". Synapse 51, 71-81.

Waddington, J.L., 1990. Spontaneous orofacial movements induced in rodents by very long-term neuroleptic drug administration: phenomenology and putative relationship to tardive dyskinesia. Psychopharmacology 101, 431-447.

Waddington, J.L., Daly, S.A., McCauley, P.G., O'Boyle, K.M., 1994. Levels of functional interaction between D_1-like and D_2-like dopamine receptor systems. In: Niznik, H.B. (Ed.), Dopamine Receptors and Transporters: Pharmacology, Structure and Function. New York, Marcel-Dekker, pp. 511-537.

Waddington, J.L., Daly, S.A., Downes, R.P., Deveney, A.M., McCauley, P.G., O'Boyle, K.M., 1995. Behavioural pharmacology of 'D_1-like' dopamine receptors: futher subtyping, new pharmacological probes and interactions with 'D_2-like' receptors. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 19, 811-831.

Waddington, J.L., Deveney, A.M., Clifford, J.J., Tighe, O., Croke, D.T., Sibley, D.R.,

Drago, J., 1998. D_1-like dopamine receptors: regulation of psychomotor behaviour, D_1-like: D_2-like interactions and effects of D_{1A} targeted gene deletion. In: Jenner, P., Demirdama, R. (Eds.), Dopamine Receptor Subtypes: From Basic Science to Clinic. Amsterdam, IOS Press, pp. 45-63.

Waddington, J.L., Clifford, J.J., McNamara, F.N., Tomiyama, K., Koshikawa, N., Croke, D.T., 2001. The psychopharmacology-molecular biology interface: exploring the behavioural roles of dopamine receptor subtypes using targeted gene deletion [‘knockout’]. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 25, 925-964.

Zhang, G.X., Sasamoto, K., 1990. Projections of two separate cortical areas for rhythmical jaw movements in the rat. Brain Res. Bull. 24, 221-230.

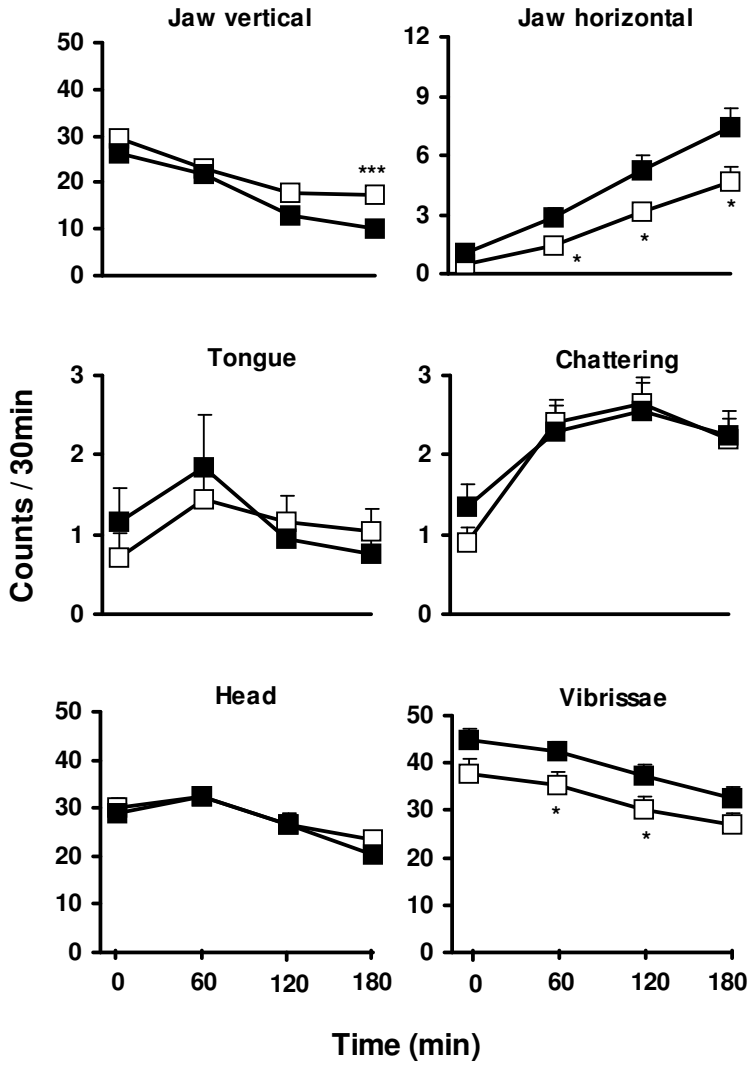

Fig. 1. Phenotype of orofacial movement topographies under restaint in male wildtypes (filled squares) and congenic D_5 mutants (open squares). Data are mean counts ± S.E.M. (n = 20 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements, over 30 min periods beginning at 0, 60, 120 and 180 min after commencing observations. Post-hoc tests: *P

< 0.05, *** $P < 0.001$ vs wildtypes.

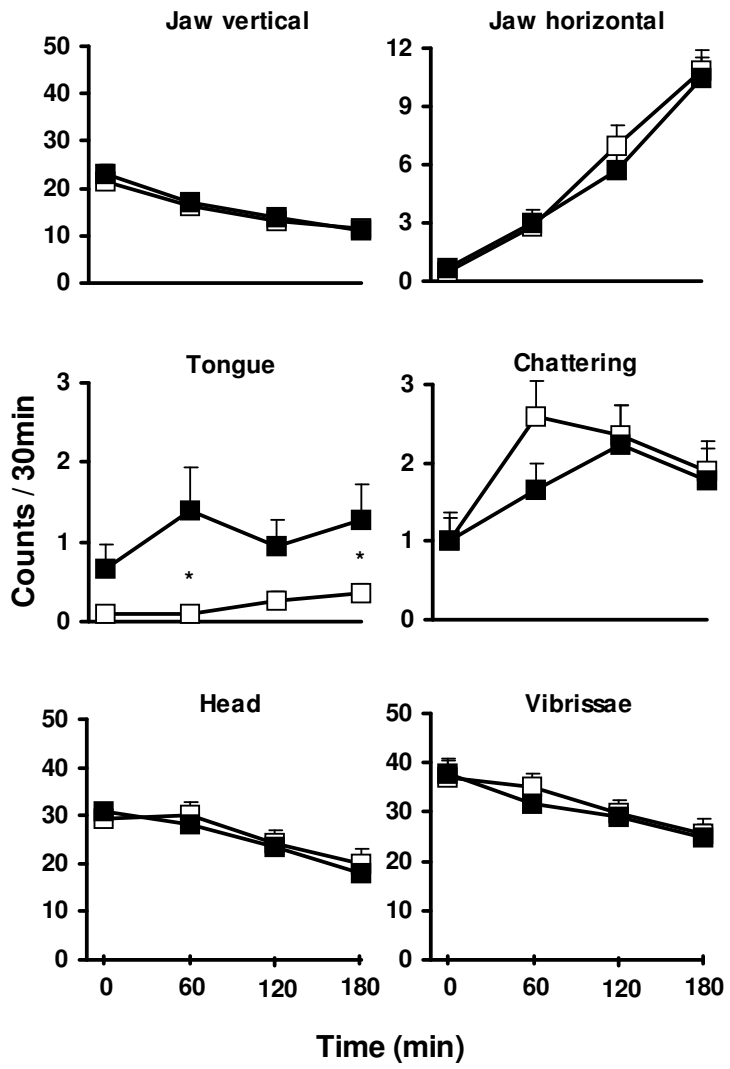

Fig. 2. Phenotype of orofacial movement topographies under restaint in male wildtypes (filled squares) and congenic DARPP-32 mutants (open squares). Data are mean counts ± S.E.M. (n = 18-20 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements, over 30 min periods beginning at 0, 60, 120 and 180 min after commencing observations. Post-hoc

test: *$P < 0.05$ vs wildtypes.

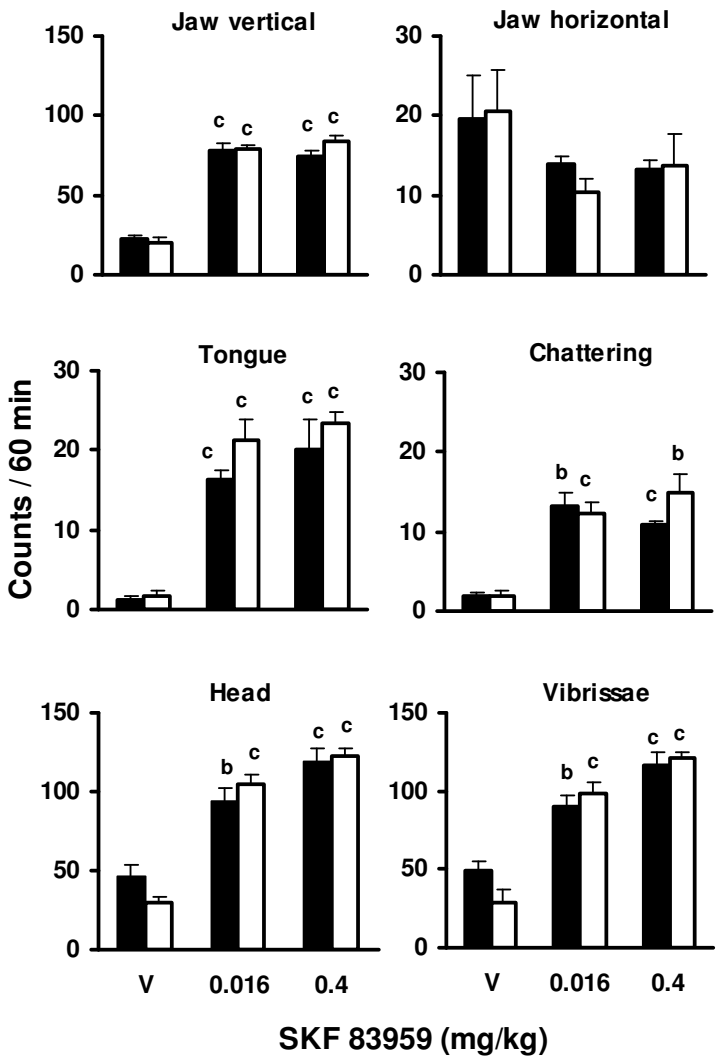

Fig. 3. Phenotype of orofacial movement topographies under restraint in wildtypes (filled columns) and congenic D_4 mutants (open columns) following challenge with 0.016- 0.4 mg/kg SK&F 83959 or vehicle (V). Data are mean counts ± S.E.M. (n = 5-7 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements, over a 60 min period. Post-hoc tests: $^{b}P < 0.01$, $^{c}P < 0.001$ vs vehicle.

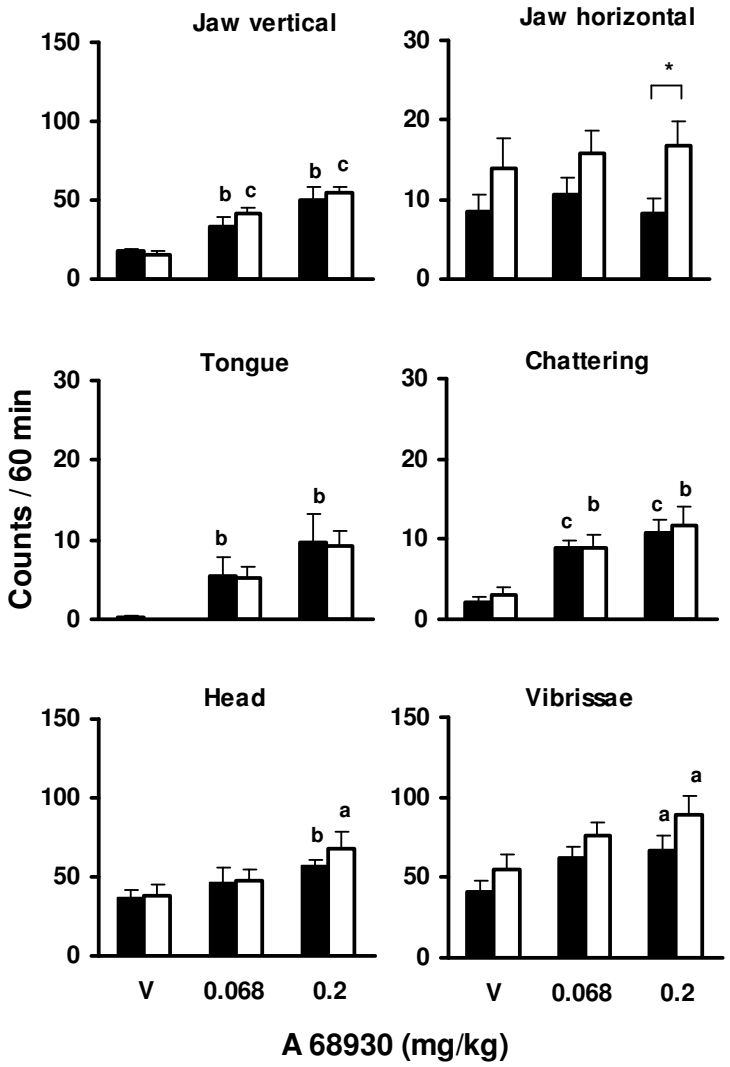

Fig. 4. Phenotype of orofacial movement topographies under restraint in wildtypes (filled columns) and congenic DARPP-32 mutants (open columns) following challenge with 0.068- 0.2 mg/kg A 68930 or vehicle (V). Data are mean counts ± S.E.M. (n = 6-7 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor

chattering, and general head and vibrissae movements, over a 60 min period. Post-hoc tests: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs vehicle; * $P < 0.05$ vs wildtypes.