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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'. figshare. Journal contribution.
<https://hdl.handle.net/10779/rcsi.10783832.v1>

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

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Abstract

The role of D₁-like [D₁, D₅] and D₂-like [D₂, D₃, D₄] dopamine receptors and dopamine transduction via DARPP-32 in topographies of orofacial movement was assessed in restrained mice with congenic D₄ vs D₅ receptor vs DARPP-32 ‘knockout’. D₄ and DARPP-32 mutants evidenced no material phenotype; also, there were no alterations in topographical responsivity to either the selective D₂-like agonist RU 24213 or the selective D₁-like agonist SK&F 83959. In contrast, D₅ 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. D₅ receptors regulate distinct topographies of vertical and horizontal jaw movement in an opposite manner. In assuming that the well-recognised role of the D₁-like family in regulating orofacial movements involves primarily D₁ receptors, a role for their D₅ counterparts may have been overlooked.

Key words: Orofacial movements; Dopamine receptors; D₄ ‘knockout’; D₅ ‘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 D₁-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 [D₁ (also known as D_{1A}), D₅ (also known as D_{1B})] has proved difficult. Specifically, in the absence of agents that can discriminate materially between D₁ and D₅ receptors, it has been assumed that the D₁ 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 D₅ receptor has a low density localisation in primarily cortical and limbic regions (Khan et al., 2000; Di Chiara, 2002).

The D₂-like family plays a lesser role in the regulation of orofacial movements, both independently and especially via D₁-like:D₂-like interactions (Waddington et al., 1994, 1995). Similarly, in the absence of agents that can discriminate materially between D₂, D₃ and D₄ receptors, it has been assumed that the D₂ receptor exerts a prepotent, if not

exclusive, role on the basis of its high density localisation in the striatum; conversely, D₃ and particularly D₄ 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 D₁-like and of D₂-like receptors, particularly in relation to D₁ and D₂ 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 D₁-like and D₂-like agonists in mice with congenic D₁, D₂ and D₃ 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 D₁-like agonist SK&F 83959 and the D₂-like agonist RU 24213, in mice with congenic D₄ vs D₅ receptor vs DARPP-32 ‘knockout’.

2. Experimental procedures

2.1. Animals

The original F2 hybrid strain [129/Ola × C57BL/6J] containing the mutated D₄ receptor allele was generated as reported previously (Rubinstein et al., 1997); animals from an essentially congenic line of D₄ ‘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 \times 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 \times 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 °C and $55 \pm 5\%$ 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 injurious 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 (Tomiya 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] (Tomiya 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 (Tomiya 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 D₂-like agonist RU 24213

[N-n-propyl-N-phenyl-*p*-3-hydroxyphenylethylamine; Hoechst-Marion-Roussel, France] was dissolved in distilled water; the selective D₁-like agonists SK&F 83959 [3-methyl-6-chloro-7,8-dihydroxy-1-[3-methyl-phenyl]-2,3,4,5-tetrahydro-1H-3-benzazepine; RBI/SRI/NIMH Chemical Synthesis Program, USA] and A 68930 [cis-(±)-1-(aminomethyl)-3,4-dihydro-3-phenyl-1H-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 \pm 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 D₄ 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 D₅ 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 D₄ mutants, this topography of orofacial movements was essentially unaltered [no effects of genotype or genotype × time interactions] (data not shown).

In congenic D₅ 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 (Tomiya et al., 2001; Makihara et al., 2004).

In congenic D₄ 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 D₄ 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 D₅ 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 \times dose interactions] (data not shown). In DARPP-32 mutants, studies with an additional D₁-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 \times 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 D₄ and D₅ members of, respectively, the D₂-like and D₁-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 D₁, D₂ and D₃ 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 (Gariépy 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 D₄ mutants evidenced no phenotypic effects in terms of orofacial movement topography under restraint. In relation to the D₂-like

family, this would complement our findings in D₂ and D₃ mutants that it is the D₂ receptor that exerts some regulatory role to inhibit vertical jaw movements, with a limited role to facilitate tongue protrusions and incisor chattering, while D₃ 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: D₂ receptors have a dense localisation within the striatum, a region known to be critical for the regulation of orofacial movements; D₃ receptors have a lower density localisation within circumscribed striatal regions and corticolimbic structures; D₄ 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 D₅ 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 D₁-like family, we have reported previously that D₁ 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 D₁ receptors have a dense localisation within the striatum and in certain cortical regions, D₅ receptors have a low density striatal and a particular corticolimbic localisation (Khan et al., 2000; Di Chiara, 2002); yet D₅ mutants exhibit phenotypic effects in terms of orofacial movement topography. Furthermore, the present differential regulatory effects of D₅ vs D₁ 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 D₁ and D₂ 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 D₁ and D₂ 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 D₄ nor D₅ mutants evidenced material alterations in topographical responsivity to the D₁-like agonist SK&F 83959 (Deveney et al., 1995; Niznik et al., 2002), which evidences similar affinities for D₁ and D₅ receptors over their D₂-like counterparts (O'Sullivan et al., 2004), or to the D₂-like agonist RU 24213 (Clifford et al., 2001; McNamara et al., 2002), which evidences similar affinities for D₂, D₃ and D₄ receptors over their D₁-like counterparts (Roth, personal communication; O'Sullivan et al., in preparation). For D₄ mutants this may reflect the limited involvement of D₄ receptors in such processes, even though a presence in the striatum is increasingly recognized (Rivera et al., 2002a), while for D₅ mutants this may reflect in part a controversy as to the functional roles of putative D₁-like receptors that are linked to a transduction mechanism other than/additional to adenylyl cyclase, and where the status of D₅ receptors remains particularly unclear (Niznik et al., 2002); in D₁ 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 D₁-like receptors linked to adenylyl cyclase (Greengard et al., 1999; Greengard, 2001), while SK&F 83959 is a D₁-like agonist which has actions at

putative D₁-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 D₁-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 D₁-like and D₂-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 D₅ mechanisms, as D₅ 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 D₁-like receptors in the regulation of orofacial movements, using pharmacological tools that cannot distinguish between D₁ and D₅ receptors (Adachi et al., 2003). Alternatively, recent evidence suggests that the presence and physiological role of D₅ 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 D₅ 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 D₁ mutants (Tomiyama et al., 2002), the present findings in D₅ mutants relate to specific orofacial movement topographies and how these change over habituation. This suggests a role for D₅ as well as for D₁ 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.

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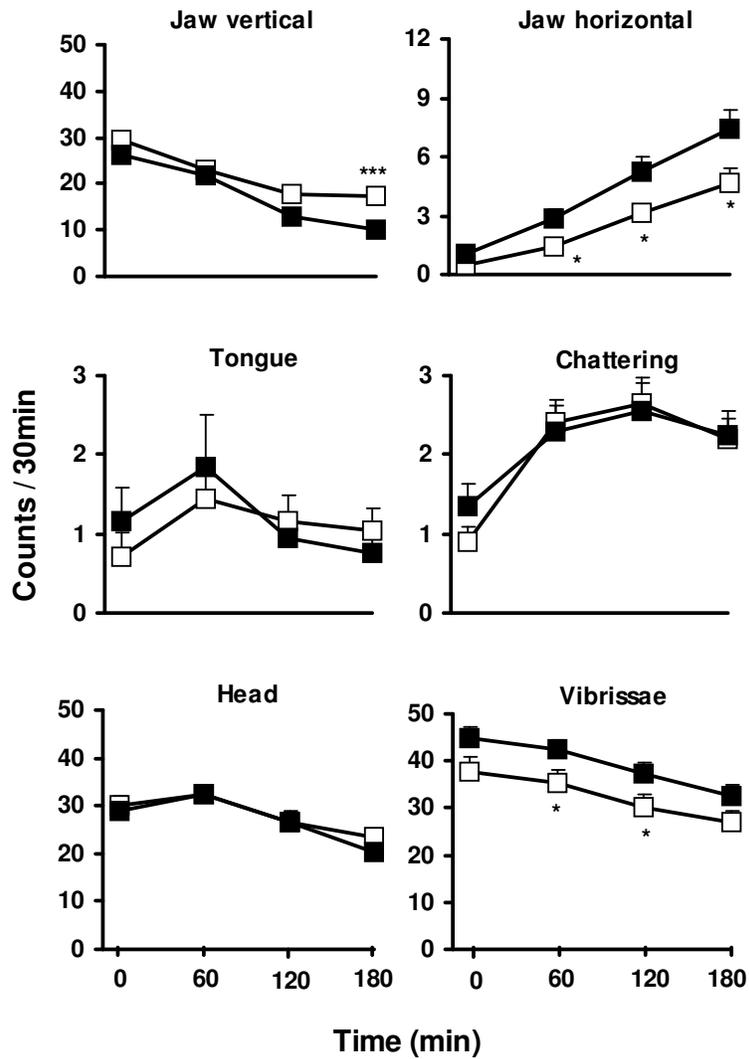


Fig. 1. Phenotype of orofacial movement topographies under restraint in male wildtypes (filled squares) and congenic D₅ mutants (open squares). Data are mean counts \pm S.E.M. (n = 20 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae 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.

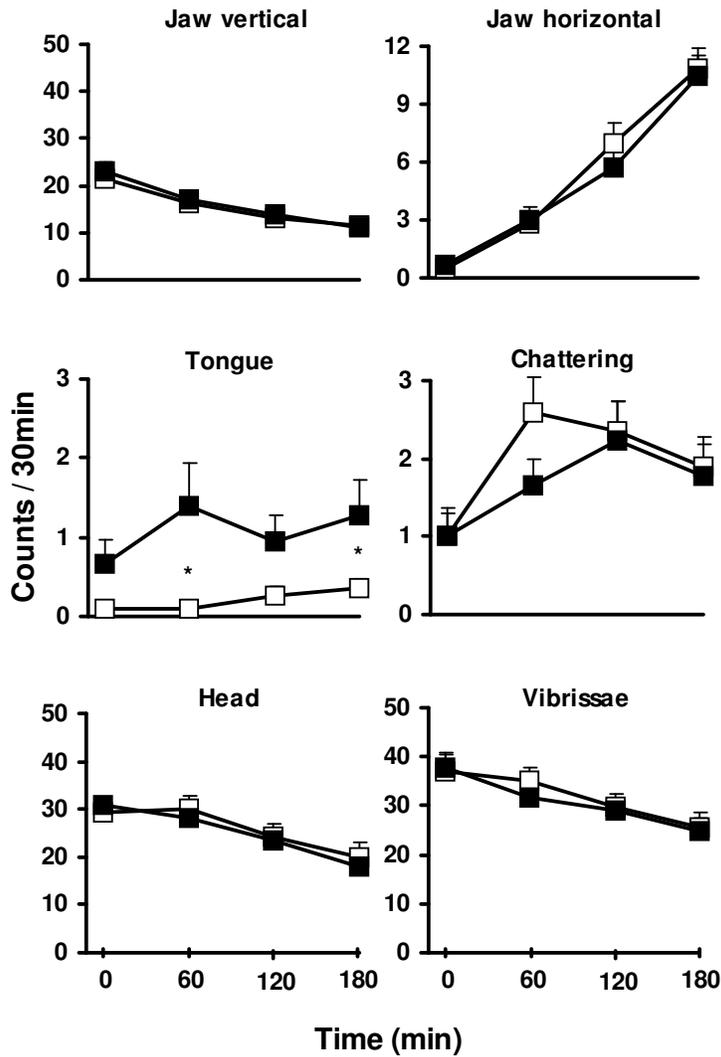


Fig. 2. Phenotype of orofacial movement topographies under restraint in male wildtypes (filled squares) and congenic DARPP-32 mutants (open squares). Data are mean counts \pm S.E.M. (n = 18-20 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head 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.

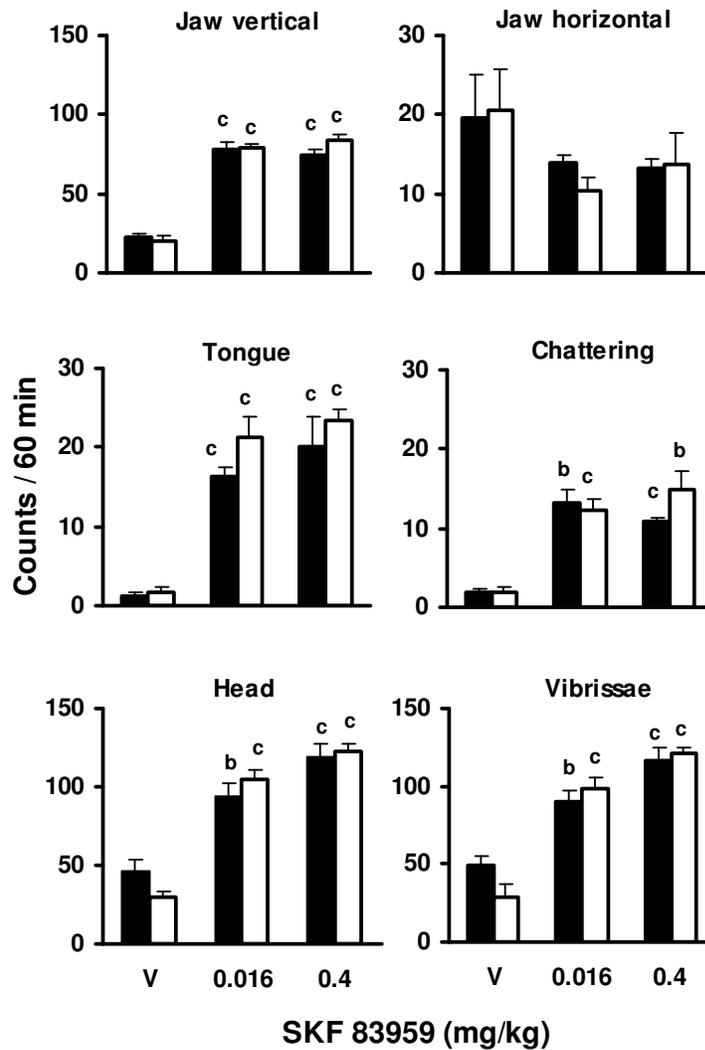


Fig. 3. Phenotype of orofacial movement topographies under restraint in wildtypes (filled columns) and congenic D₄ mutants (open columns) following challenge with 0.016- 0.4 mg/kg SK&F 83959 or vehicle (V). Data are mean counts \pm S.E.M. (n = 5-7 per group) for vertical and horizontal jaw movements, tongue protrusions, incisor chattering, and general head and vibrissae movements, over a 60 min period. Post-hoc tests: ^bP < 0.01, ^cP < 0.001 vs vehicle.

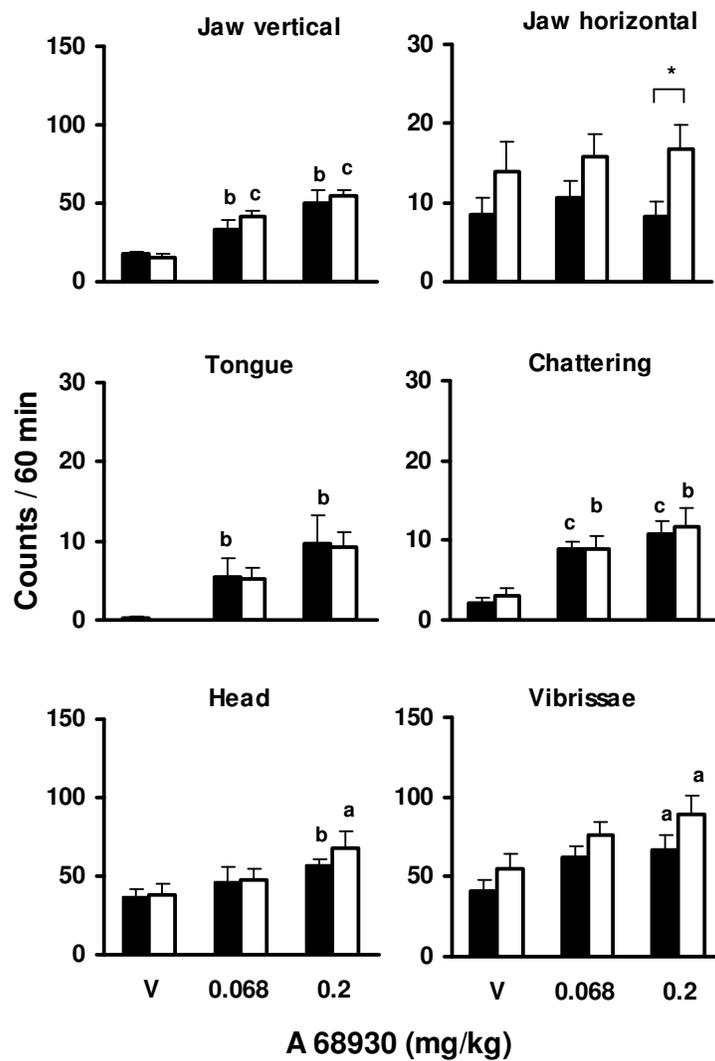


Fig. 4. Phenotype of orofacial movement topographies under restraint in wildtypes (filled columns) and congenic DARPP-32 mutants (open columns) following challenge with 0.068- 0.2 mg/kg A 68930 or vehicle (V). Data are mean counts \pm S.E.M. (n = 6-7 per group) for vertical and horizontal jaw movements,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

chattering, and general head and vibrissae movements, over a 60 min period. Post-hoc tests: ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 vs vehicle; * P < 0.05 vs wildtypes.