

Ethologically based resolution of D₂-like dopamine receptor agonist- vs antagonist-induced behavioural topography in DARPP-32 ‘knockout’ mutants congenic on the C57BL/6 genetic background

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a) Running title: D₂-like receptor ethogram in congenic DARPP-32 mutants

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c) Number of text pages: 25

Number of tables: 0

Number of figures: 4

Number of references: 31

Number of words in *Abstract*: 246

Number of words in *Introduction*: 738

Number of words in *Discussion*: 1130

d) Abbreviations: DARPP-32, dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32 kilodaltons; RU 24213, N-*n*-propyl-N-phenylethyl-*p*-3-hydroxyphenylethylamine; YM 09151-2, *cis*-N-[1-benzyl-2-methyl-pyrrolidin-3-yl]-5-chloro-2-methoxy-4-methylaminobenzamide; PP-1, protein phosphatase-1; PKA, protein kinase A

e) Recommended section assignment: Behavioural Pharmacology

ABSTRACT

Given the critical role of DARPP-32 in the regulation of dopaminergic function, DARPP-32-null mutant mice congenic on the inbred C57BL/6 strain for 10 generations were examined phenotypically for their *ethogram* of responsivity to the selective D₂-like receptor agonist RU 24213 and the selective D₂-like receptor antagonist YM 09151-2, using procedures which resolve all topographies of behaviour in the natural repertoire. Following vehicle challenge, levels of sniffing and rearing seated were reduced in DARPP-32 mutants; the injection procedure appears to constitute a 'stressor' that reveals phenotypic effects of DARPP-32 deletion not apparent under naturalistic conditions. Topographical effects of 0.3-10.0 mg/kg RU 24213, primarily induction of sniffing and ponderous locomotion with accompanying reductions in rearing, grooming, sifting and chewing, were not altered to any material extent in DARPP-32-null mice. However, topographical effects of 0.005-0.625 mg/kg YM 09151-2, namely reduction in sniffing, locomotion, rearing, grooming and chewing but not sifting, were essentially absent in DARPP-32 mutants. Thus, the D₂-like receptor agonist-mediated *ethogram* was essentially conserved, while major elements of the corresponding D₂-like receptor antagonist-mediated *ethogram* were essentially absent in DARPP-32-null mice. This suggests some relationship between (i) extent of tonic dopaminergic activation of DARPP-32 mechanisms and (ii) compensatory mechanisms consequent to the developmental absence of DARPP-32 which may emerge to act differentially on individual elements of the DARPP-32 system. Critically, the present data indicate that phenotypic effects of a given gene deletion using an agonist acting on the system disrupted cannot be generalised to a corresponding antagonist, and vice-versa.

Investigation of the roles of individual D₁-like [D₁, D₅] and D₂-like [D_{2L/S}, D₃, D₄] dopamine receptor subtypes in the regulation of mammalian behaviour remains uncertain because of delay in the identification of a full range of selective agonists and antagonists by medicinal chemistry relative to the rate of identification of these receptor subtypes by molecular biology (Waddington et al., 1995; Di Chiara, 2002; Sidhu et al., 2003). The construction of mutants with targeted gene deletion ('knockout') of each individual dopamine receptor subtype has provided an important approach to addressing this issue (Sibley, 1999; Waddington et al., 2001). However, elucidation of the cellular mechanisms by which events at these subtypes are transduced to ultimately influence behaviour presents yet greater challenges.

Integral to these processes is dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein of 32 kilodaltons (DARPP-32). In response to dopamine, this neuronal phosphoprotein is converted into a potent inhibitor of protein phosphatase-1 (PP-1; Hemmings et al., 1984), a critical determinant of the state of phosphorylation and hence the physiological activity of a wide array of neuronal phosphoproteins, including neurotransmitter receptors, ion channels and transcription factors. At this cellular level, D₁-like receptors activate the phosphorylation of DARPP-32, via adenylyl cyclase and protein kinase A (PKA), to inhibit PP-1, while D₂-like receptors dephosphorylate DARPP-32, both via inhibition of PKA and through an adenylyl cyclase-independent pathway, to disinhibit PP-1; thus, DARPP-32 is regarded as an essential mediator of the biological effects of DA (Greengard et al., 1999). To investigate further its functional role in the absence of specific pharmacological tools, DARPP-32-null mice have been constructed (Fienberg et al., 1998). The cellular phenotype of these mutants confirms deletion of functional DARPP-32 in the absence of changes in the number of D₁-like or D₂-like receptors (Fienberg and Greengard, 2000; Svenningsson et al., 2000).

In relation to dopamine-mediated function, studies in mutants present both opportunities and challenges. At the level of behaviour, assessment of otherwise undifferentiated ‘activity’ in terms of photobeam interruptions, or observational assessments restricted to operational definitions of gross elements of behaviour, over limited time-frames, can obscure critical phenotypic effects; these can be addressed by application of ethologically-based approaches which resolve all topographies of behaviour within the mouse repertoire [i.e. specification of its *ethogram*] over the prolonged time course of exploration of and subsequent habituation to its environment (Waddington et al., 2001; McNamara et al., 2003). Additionally, the mixed [129/Sv × C57BL/6] genetic background, on which essentially all dopamine-related ‘knockouts’ have been constructed and examined to date, leaves open the possibility that phenotypic effects might reflect not only the entity deleted but also variations in that genetic background (Kelly et al., 1998; Phillips et al., 1999; Waddington et al., 2001); this potential problem can be overcome in substantial part by repeated back-crossing onto a single strain, usually C57BL/6, to attain essential congenicity (Banbury Conference, 1997; Tomiyama et al., 2002; McNamara et al., 2002, 2003).

On this basis, we have recently studied congenic DARPP-32 mutants at an ethological level, with the resultant *ethogram* revealing only subtle phenotypic effects at the level of spontaneous behaviour (Nally et al., 2003). In initial studies with dopaminergic agents, the acute stimulatory response to the non-selective, indirect dopaminergic agonist amphetamine, and catalepsy induced by lower but not higher doses of the antipsychotic raclopride, appeared reduced in DARPP-32 mutants (Fienberg and Greengard, 2000; Svenningsson et al., 2003). In our own studies (Nally et al., 2003), we reported ethologically-based alterations in the topography of behavioural responsivity to the non-selective dopamine receptor agonist apomorphine in DARPP-32 mutants. However, the role of DARPP-32 in mediating distinct

topographies of D₂-like-mediated behaviour, as induced by selective stimulation of D₂-like receptors and attenuated by selective antagonism thereof, remains to be clarified.

Described here are experiments to resolve ethologically the congenic DARPP-32 mutant phenotype following such specific pharmacological manipulation of D₂-like receptor systems. These studies utilise procedures which both address the above methodological concerns and, additionally, allow systematic comparison with the phenotype of congenic D₁, D₂ and D₃ receptor 'knockouts' which we have determined using these same procedures (Clifford et al., 2001; McNamara et al., 2002, 2003). They contrast the effects of the selective D₂-like receptor agonist RU 24213 (Euvrard et al., 1980; Clifford et al., 2000, 2001; McNamara et al., 2002, 2003) with those of the selective D₂-like receptor antagonist YM 09151-2 (Niznik et al., 1985; McNamara et al., 2003), to evaluate shifts in the topography of behaviour mediated through D₂-like receptors, consequent to the absence of DARPP-32.

Materials and Methods

Targeted Gene Deletion. The generation of DARPP-32 ‘knockout’ mice was as reported previously (Fienberg et al., 1998). In outline, the targeted gene deletion was constructed in 129/Ola-derived embryonic stem cells and male chimeras mated with C57BL/6J females to produce heterozygous mutants (DARPP-32^{+/-}); these were then backcrossed into C57BL/6J for 10 generations to create a congenic DARPP-32-null line. Congenic, homozygous (DARPP-32^{-/-}) and wildtype (DARPP-32^{+/+}) breeding pairs were then transported to Dublin, where homozygous mutants were generated from homozygous mutant breeding pairs, while wildtypes were generated from wildtype breeding pairs; the genotype of all progeny was confirmed using PCR analysis of isolated tail DNA. Animals were housed in groups of 4-5, with food and water available *ad libitum*, and maintained at 21 ± 1°C on a 12/12 hr (08:00 on; 20:00 off) light/dark schedule. Young adult mice from litters of the same generational age were used in all studies. These studies were approved by the Research Ethics Committee of the Royal College of Surgeons in Ireland and were conducted under license from the Department of Health & Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals.

Drugs. RU 24213 [N-n-propyl-N-phenylethyl-p-3-hydroxyphenylethylamine; Hoechst-Marion-Roussel, France] was dissolved in distilled water; YM 09151-2 [*cis*-N-(1-benzyl-2-methyl-pyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide; Yamanouchi, Japan] was dissolved in 0.1 N HCl and made up to volume with distilled water. Drugs and their respective vehicles were injected subcutaneously into the flank in a volume of 2 ml/kg.

Behavioural Assessment. On experimental days mice were removed from their home cage and placed individually in clear glass observation cages (36 × 20 × 20 cm). Mice were allowed to habituate to observation cages for a period of 3 h, to limit topographies of initial exploration before comparison of D₂-like receptor agonist and antagonist effects.

Behavioural assessments were carried out using a rapid time-sampling behavioural checklist technique, in a manner similar to that described previously for congenic D₁, D₂ and D₃ receptor mutants given RU 24213 and/or YM-09151-2 (Clifford et al., 2001; McNamara et al., 2002, 2003) and for DARPP-32 mutants given apomorphine (Nally et al., 2003).

Immediately following drug challenge, each of ten randomly allocated mice was observed individually for 5 s periods at 1 min intervals over 15 consecutive minutes using an extended, ethologically-based behavioural checklist. This allowed the presence or absence of the following individual behaviours (occurring alone or in combination) to be determined in each 5 s period: sniffing (flaring of nostrils with movement of vibrissae); locomotion (coordinated movement of all four limbs producing a change in location); ponderous locomotion, a 'plodding' variant induced in mice by D₂-like receptor agonists that differs from normal, fluid ambulation (Clifford et al., 1999, 2000, 2001; McNamara et al., 2002, 2003); total rearing (rearing of any form); rearing seated (front paws reaching upwards with hind limbs on floor in sitting position); rearing free (front paws reaching upwards away from any cage wall while standing on hind limbs); rearing to wall (front paws reaching upwards onto or towards a cage wall while standing on hind limbs); sifting (characteristic sifting movements of the front paws through bedding material on cage floor); grooming (of any form); intense grooming (characteristic pattern of grooming of the snout and then the face with the forepaws, followed by vigorous grooming of the hind flank or anogenital region with the snout); chewing (chewing movements directed onto cage bedding and/or fecal pellets without consumption); stillness (motionless, with no behaviour evident).

This cycle of assessment by behavioural checklist over a 15 min period (0-15 min) was repeated twice (20-35 min and 40-55 min). For evaluation of agonist/antagonist-induced behaviour, mice were used on two occasions only, separated by a drug-free interval of at least one week; on each occasion mice were allocated randomly to one of the various treatments. All assessments were made by an observer who was unaware of genotype and treatment for each animal.

Data Analysis. For drug-related *ethograms*, the total ‘counts’ for each individual topography of behaviour was determined as the number of 5 s observation windows in which a given behaviour was evident, summed over the initial 3×15 min (0-15, 20-35, 40-55 min) cycle periods, and expressed as means \pm S.E.M. ‘Counts’ for individual behaviours in relation to drug dose and genotype were analysed using analysis of variance (ANOVA) following square root transformation (Ross et al., 2000; McNamara et al., 2002, 2003; Tomiyama et al., 2002, 2004; Nally et al., 2003), to allow examination of interaction effects in the absence of non-parametric techniques for interaction terms, followed by Student’s t-test.

Results

General Parameters: RU 24213 Challenge. On examining 19 female congenic DARPP-32-null mice, mean body weight [22 ± 1 g, mean age 127 ± 6 days] did not differ relative to 20 female wildtypes [22 ± 1 g, mean age 136 ± 9 days]. On qualitative inspection of posture, reactivity to handling, and general activity, no gross motor phenotype was apparent, in agreement with our previous report (Nally et al., 2003).

Ethogram of Responsivity to RU 24213. Administration of RU 24213 (0.3–10.0 mg/kg; Figs. 1 and 2) exerted a biphasic effect on sniffing: there was inhibition at lower doses and stimulation at higher doses (effect of dose, $F_{4,70} = 9.19$, $p < 0.001$); overall levels of sniffing were reduced in congenic DARPP-32-null mice throughout the dose range (effect of genotype, $F_{1,70} = 11.44$, $p < 0.005$; no dose \times genotype interaction); in particular, sniffing was reduced in vehicle-treated DARPP-32 mutants ($p < 0.05$). Rearing topographies were inhibited by increasing doses of RU 24213, among which rearing seated was the primary component (effect of dose, $F_{4,70} = 3.18$, $p < 0.05$); overall levels of rearing seated were reduced in DARPP-32 mutants throughout the dose range (effect of genotype, $F_{1,70} = 14.75$, $p < 0.001$; no dose \times genotype interaction); in particular, rearing seated was reduced in vehicle-treated DARPP-32-null mice ($p < 0.01$). Levels of rearing free and rearing to wall were too low for meaningful analysis (data not shown). These doses of RU 24213 exerted a polyphasic effect on locomotion: there was inhibition of locomotion at lower doses, which reversed to normal levels at an intermediate dose, before diminishing again at the highest dose with transition to ponderous locomotion (effect of dose, $F_{4,70} = 7.90$, $p < 0.001$); overall levels of locomotion were reduced in DARPP-32 mutants throughout the dose range (effect of genotype, $F_{1,70} = 4.35$, $p < 0.05$; no dose \times genotype interaction); ponderous locomotion was

induced in a dose-dependent manner that did not differ between the genotypes (effect of dose, $F_{4,70} = 11.28$, $p < 0.001$; no effect of genotype or dose \times genotype interaction).

Grooming topographies were inhibited by increasing doses of RU 24213 (effect of dose, $F_{4,70} = 12.72$, $p < 0.001$), and this effect did not differ between the genotypes (no effect of genotype or dose \times genotype interaction); levels of intense grooming syntax were too low for meaningful analysis. Sifting was inhibited similarly (effect of dose, $F_{4,70} = 10.09$, $p < 0.001$), though this inhibition was diminished in DARPP-32 mutants at an intermediate dose of RU 24213 (dose \times genotype interaction, $F_{4,70} = 2.68$, $p < 0.05$). Chewing was inhibited by RU 24213 throughout this dose range, in a manner that did not differ between the genotypes (effect of dose, $F_{4,70} = 10.94$, $p < 0.001$; no effect of genotype or dose \times genotype interaction).

These doses of RU 24213 exerted a biphasic effect on stillness: as this category integrates the absence of the above topographies of behaviour, it was increased at lower doses and decreased at higher doses (effect of dose, $F_{4,70} = 9.76$, $p < 0.001$); in keeping with overall reductions in levels of sniffing, rearing seated and locomotion, overall levels of stillness were increased in DARPP-32 mutants throughout the dose range (effect of genotype, $F_{1,70} = 13.69$, $p < 0.001$; no dose \times genotype interaction); in particular, stillness was increased in vehicle-treated DARPP-32 mutants ($p < 0.05$) which paralleled decreased levels of sniffing and rearing seated under this condition.

General Parameters: YM 09151-2 Challenge. On examining 20 female congenic DARPP-32-null mice, mean body weight [22 ± 1 g, mean age 176 ± 10 days] did not differ relative to 20 female wildtypes [23 ± 1 g, mean age 188 ± 6 days].

Ethogram of responsivity to YM 09151-2. In wildtypes, YM 09151-2 (0.005 – 0.625 mg/kg; Figs. 3 and 4) readily and dose-dependently reduced sniffing and rearing topographies, among which rearing seated was the primary component, but was without effect in DARPP-32-null mice (sniffing: effect of dose, $F_{4,70} = 8.35, p < 0.001$; dose \times genotype interaction, $F_{4,70} = 7.82, p < 0.001$; rearing seated: effect of dose, $F_{4,70} = 10.57, p < 0.001$; dose \times genotype interaction, $F_{4,70} = 5.21, p < 0.005$); there were reduced levels of sniffing and rearing seated in vehicle-treated DARPP-32 mutants ($p < 0.05$) which, because of this lack of effect of YM 09151-2, endured across its dose range to ultimately exceed ($p < 0.05$) that in wildtypes receiving higher doses. Levels of rearing free and rearing to wall were too low for meaningful analysis (data not shown). There was also a dose-dependent reduction in locomotion, which did not differ between the genotypes (effect of dose, $F_{4,70} = 4.16, p < 0.005$; no effect of genotype or dose \times genotype interaction); ponderous locomotion was absent.

In wildtypes, YM 09151-2 readily and dose-dependently reduced grooming topographies but was without effect in DARPP-32-null mice, such that overall levels in mutants exceeded those in wildtypes (effect of dose, $F_{4,70} = 10.51, p < 0.001$; effect of genotype, $F_{1,70} = 4.87, p < 0.05$; dose \times genotype interaction, $F_{4,70} = 3.32, p < 0.05$); levels of intense grooming syntax were too low for meaningful analysis. Low levels of sifting showed an overall increase in DARPP-32 mutants (effect of genotype, $F_{1,70} = 7.47, p < 0.01$; no effect of dose or dose \times genotype interaction), while low levels of chewing was unaltered (no effect of genotype, dose or dose \times genotype interaction).

These doses of YM 09151-2 readily and dose-dependently increased levels of stillness in wildtypes but were without effect in DARPP-32-null mice (effect of dose, $F_{4,70} = 8.88, p < 0.001$; dose \times genotype interaction, $F_{4,70} = 5.51, p < 0.005$). DARPP-32 mutants evidenced an increased level of stillness relative to wildtypes following vehicle treatment ($p < 0.05$) which paralleled their decreased levels of sniffing and rearing seated, and a decreased level of

stillness relative to wildtypes following treatment with high doses of YM 09151-2 ($p < 0.01$) which paralleled their increased levels of sniffing and rearing seated.

Discussion

Selective stimulation of the D₂-like dopamine receptor family with RU 24213 resulted in a characteristic behavioural profile in wildtypes: lower doses inhibited the topographies of sniffing, locomotion, sifting, chewing and grooming, while higher doses induced a transition from normal, fluid locomotion to ‘ponderous’ locomotion with sniffing, in association with further reduction in rearing, sifting, chewing and grooming. Stillness is a category that integrates the absence of any activities. This was increased by lower doses of RU 24213, in keeping with reductions in the above topographies of behaviour via stimulation of presynaptic D₂-like autoreceptors or inhibitory postsynaptic D₂-like receptors (Levant, 1977; Clifford and Waddington, 1998); conversely, stillness was decreased by higher doses of RU 24213, in keeping with increases in the above topographies of behaviour via stimulation of postsynaptic D₂-like receptors which synergise with endogenous dopaminergic transmission through D₁-like receptors in their genesis (Waddington et al., 1994, 1995). This profile is in accordance with our previous studies in wildtype counterparts of congenic D₁ (McNamara et al., 2003), D₂ (Clifford et al., 2001) and D₃ (McNamara et al., 2002) receptor mutants.

These topographical effects of RU 24213 were not altered to any material extent in DARPP-32-null mice, which thus appeared to evidence an essentially unaltered *ethogram* in response to selective stimulation of D₂-like receptors in the absence of DARPP-32. Activation of D₂-like receptors activates the dephosphorylation of DARPP-32 at Thr-34 and disinhibition of PP-1 via two synergistic mechanisms: (i) inhibition of D₁-like receptor-mediated stimulation of adenylyl cyclase and PKA, and (ii) increase in intracellular calcium, which activates calcium-dependent protein phosphatase-2B (Greengard, 2001). As the phosphorylation state of DARPP-32 at Thr-34 and the degree of inhibition of PP-1 is a critical regulator of dopamine-mediated function (Greengard et al., 1999; Greengard, 2001), the

present 'normal' D₂-like receptor agonist-mediated *ethogram* in the absence of DARPP-32 appears paradoxical. While explanations in terms of mutant genetic background, insensitivity of the ethologically-based techniques employed, or over-interpretation of the functional role of DARPP-32 do not appear likely, other possibilities such as the operation of compensation processes consequent to the developmental absence of DARPP-32 should be considered; these issues have been discussed in detail elsewhere (Nally et al., 2003). Also, as in all such mutant studies, some influence of modifier genes associated with the DARPP-32 locus may carry with the mutation over repeated back-crosses to influence the resultant phenotype.

However, it must be emphasised that targeted gene deletion of DARPP-32 is not phenotypically 'silent'. In particular, following vehicle challenge levels of sniffing and rearing seated were reduced, and levels of stillness increased, in DARPP-32 mutants; yet under non-challenge conditions, such mutants evidenced little or no change in these topographies of behaviour (Nally et al., 2003). It appears that the injection procedure constitutes a 'stressor' that is able to reveal topographically specific phenotypic effects of DARPP-32 deletion that are not apparent under more naturalistic conditions; thus DARPP-32, or at least the developmental absence of DARPP-32, may interact importantly with the biological substrate(s) of this and potentially other stress-related processes in the regulation of individual topographies of behaviour. Acute, unavoidable stress is associated with increased release of dopamine (Deutch and Roth, 1990; Kalivas and Duffy, 1995). This may be a component of the vehicle-challenge aspect of the DARPP-32-null phenotype and of previously reported phenotypic effects of DARPP-32 deletion such as reduction in otherwise undifferentiated 'repetitive movements' in response to the non-selective dopamine-releasing agent amphetamine (Svenningsson et al., 2003).

Furthermore, there was an overall reduction in levels of sniffing, rearing seated and locomotion in DARPP-32 mutants that was unrelated to the dose of RU 24213 administered,

and which echoes the similar effect noted in our previous study with apomorphine (Nally et al., 2003). This emphasises that the phenotype of DARPP-32 deletion at the level of behaviour is accentuated under stressful relative to non-stressful conditions, in a topographically specific manner, and is manifest even under conditions of D₂-like receptor agonist-induced activation. It should be noted that the above phenotypic effects were assessed in female mutants. In our initial studies on the phenotype of DARPP-32-null mice at the level of the topography of spontaneous behaviour, gender-specific effects were encountered. As these related to phenotypic effects expressed in females but not in males (Nally et al., 2003), our previous drug challenge studies with apomorphine and the present studies with RU 24213 and YM 09151-2 utilised females. It would be necessary to conduct similar drug challenge studies in males before such findings could be generalised unequivocally across the genders.

However, a yet greater paradox is apparent. While this D₂-like receptor agonist-mediated *ethogram* was essentially conserved, the corresponding D₂-like receptor antagonist-mediated *ethogram* was altered substantively in the absence of DARPP-32; this echoes in part a previous note that catalepsy induced by lower, but not higher, doses of the D₂-like receptor antagonist raclopride appeared reduced in DARPP-32 mutants (Feinberg et al., 1998). Here, the *ethogram* of the selective D₂-like receptor antagonist YM 09151-2 to readily reduce sniffing, rearing seated and grooming in wildtypes, as described previously for wildtype counterparts of congenic D₁ mutants (McNamara et al., 2003), was essentially absent in DARPP-32 mutants; these effects appeared topographically specific, in that locomotion evidenced an intermediate phenotype while low levels of chewing and sifting evidenced an alternative phenotype. In particular, following vehicle challenge levels of sniffing and rearing seated were again reduced and levels of stillness were increased in DARPP-32 mutants; these altered baseline levels remained entirely uninfluenced by pre-treatment with any dose of YM 09151-2.

How might this paradox be resolved? As for the great majority of such studies, though RU 24213 and YM 09151-2 are recognised as 'selective' D₂-like receptor agonists and antagonists, respectively (see Introduction), we cannot exclude incontrovertibly some influence from as yet unknown properties of these agents. More specifically, for the D₂-like receptor agonist-mediated *ethogram* to be essentially conserved but with the major elements of the corresponding D₂-like receptor antagonist-mediated *ethogram* essentially absent in DARPP-32-null mice argues against explanation in terms of a unitary compensatory mechanism consequent to the developmental absence of DARPP-32. Rather, this profile suggests some relationship to extent of tonic dopaminergic activation of DARPP-32-related mechanisms. It has been argued that *in vivo* DARPP-32 phosphorylation is kept low by tonic dopaminergic activation of D₂-like receptors (Svenningsson et al., 2000). Additionally, compensatory mechanisms consequent to the developmental absence of DARPP-32 (Nally et al., 2003) may emerge to varying extents to act differentially on individual elements of the DARPP-32/PP-1 regulatory system. An interaction between such processes might influence the phenotypic effect of DARPP-32 deletion towards reduced consequence for D₂-like receptor agonist and greater consequence for D₂-like receptor antagonist effects. Independent of such considerations, the present data indicate critically that the phenotypic effects of a given targeted gene deletion obtained using an agonist acting on the system disrupted cannot be generalised to a corresponding antagonist, and vice-versa.

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This work was supported by [J.L.W.] Science Foundation Ireland - Enterprise Ireland, the Stanley Medical Research Institute, and a Galen Fellowship from the Irish Brain Research Foundation, in the Institute of Biopharmaceutical Science under the Higher Education Authority's Programme for Research in Third Level Institutions, and by [P.G.] DA 10044 and MH 39327.

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Fig. 1. Topographical effects of pretreatment with 0.3 – 10.0 mg/kg RU 24213 or vehicle (V). Data are mean behavioural counts \pm S.E.M. over a 60 min period for $n = 7-8$ per group for sniffing, rearing seated, locomotion and ponderous locomotion in wildtype (closed columns) vs DARPP-32-null (open columns) female mice. $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$, $^{\text{†††}}p < 0.001$ vs vehicle-treated control of the same genotype; $*p < 0.05$, $**p < 0.01$ between genotypes receiving the same dose.

Fig. 2. Topographical effects of pretreatment with 0.3 – 10.0 mg/kg RU 24213 or vehicle (V). Data are mean behavioural counts \pm S.E.M. over a 60 min period for $n = 7-8$ per group for grooming, sifting chewing and stillness in wildtype (closed columns) vs DARPP-32-null (open columns) female mice. $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$, $^{\text{†††}}p < 0.001$ vs vehicle-treated control of the same genotype; $*p < 0.05$, $**p < 0.01$ between genotypes receiving the same dose.

Fig. 3. Topographical effects of pretreatment with 0.005 – 0.625 mg/kg YM 09151-2 or vehicle (V). Data are mean behavioural counts \pm S.E.M. over a 60 min period for $n = 8$ per group for sniffing, rearing seated, locomotion and ponderous locomotion in wildtype (closed columns) vs DARPP-32-null (open columns) female mice. $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$, $^{\text{†††}}p < 0.001$ vs vehicle-treated control of the same genotype; $*p < 0.05$, $**p < 0.01$ between genotypes receiving the same dose.

Fig. 4. Topographical effects of pretreatment with 0.005 – 0.625 mg/kg YM 09151-2 or vehicle (V). Data are mean behavioural counts \pm S.E.M. over a 60 min period for $n = 8$ per group for grooming, sifting chewing and stillness in wildtype (closed columns) vs DARPP-32-null (open columns) female mice. $^{\dagger}p < 0.05$, $^{\text{†††}}p < 0.001$ vs vehicle-treated control of the same genotype; $*p < 0.05$, $**p < 0.01$ between genotypes receiving the same dose.

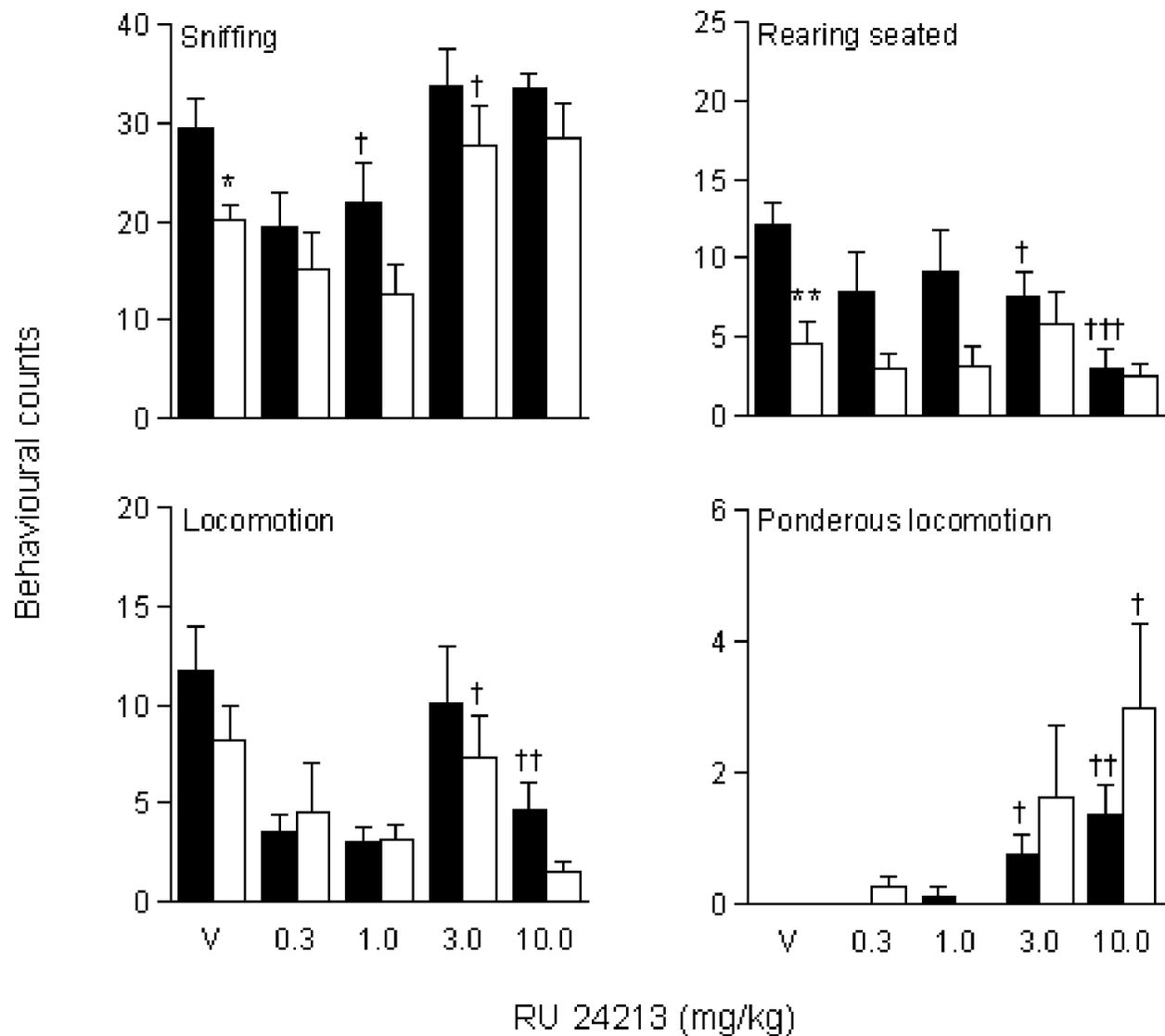


Figure 1

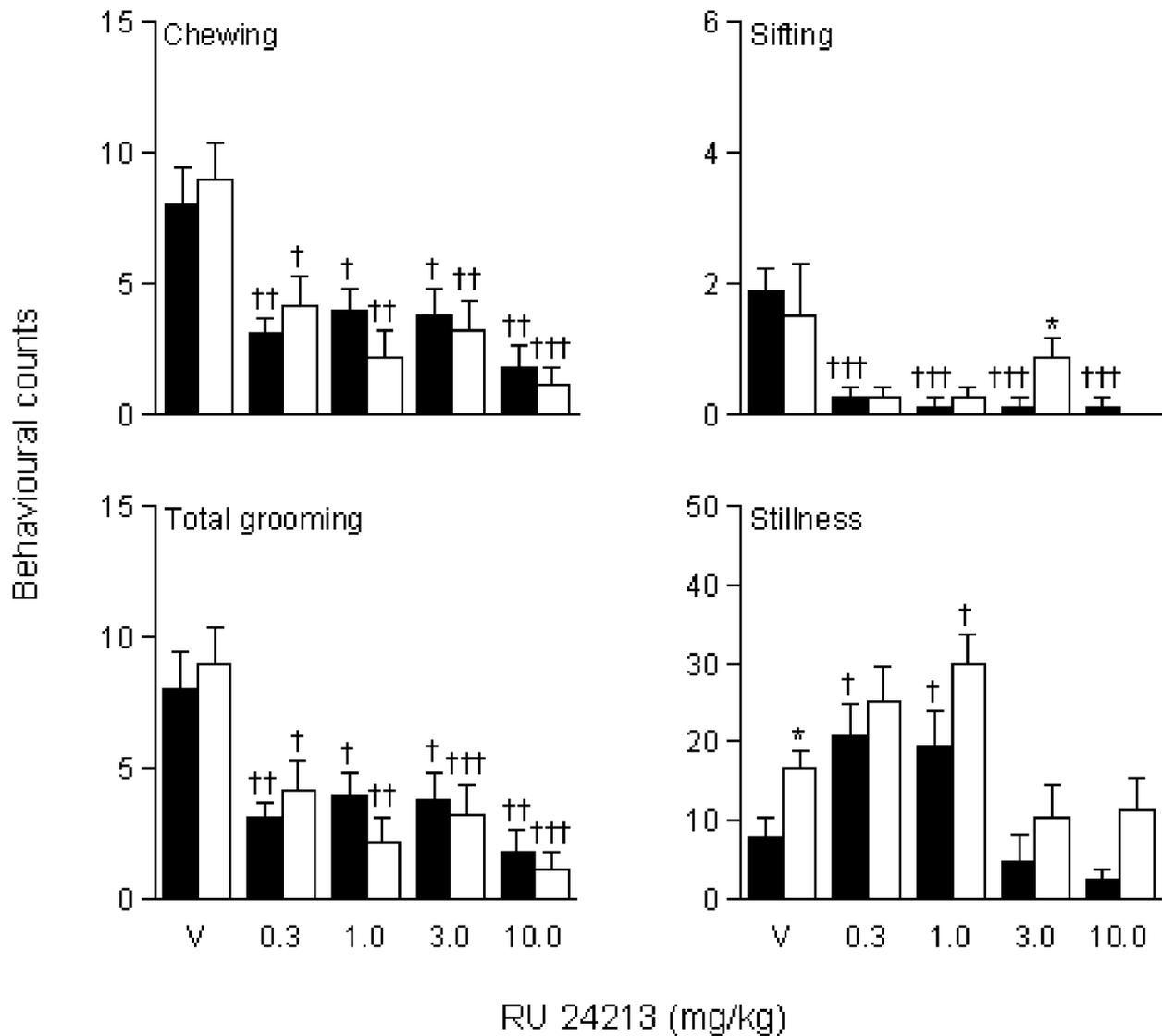


Figure 2

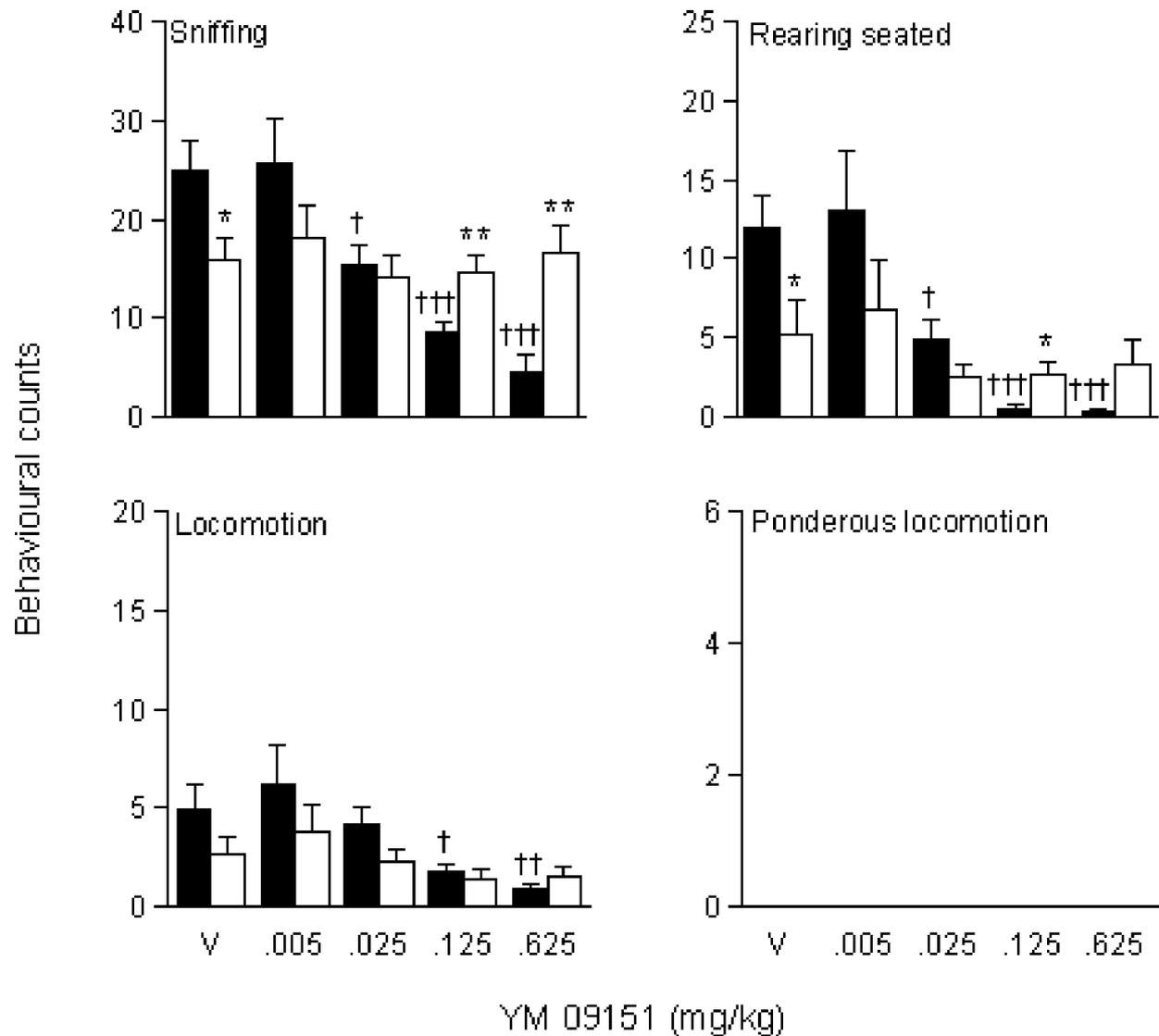


Figure 3

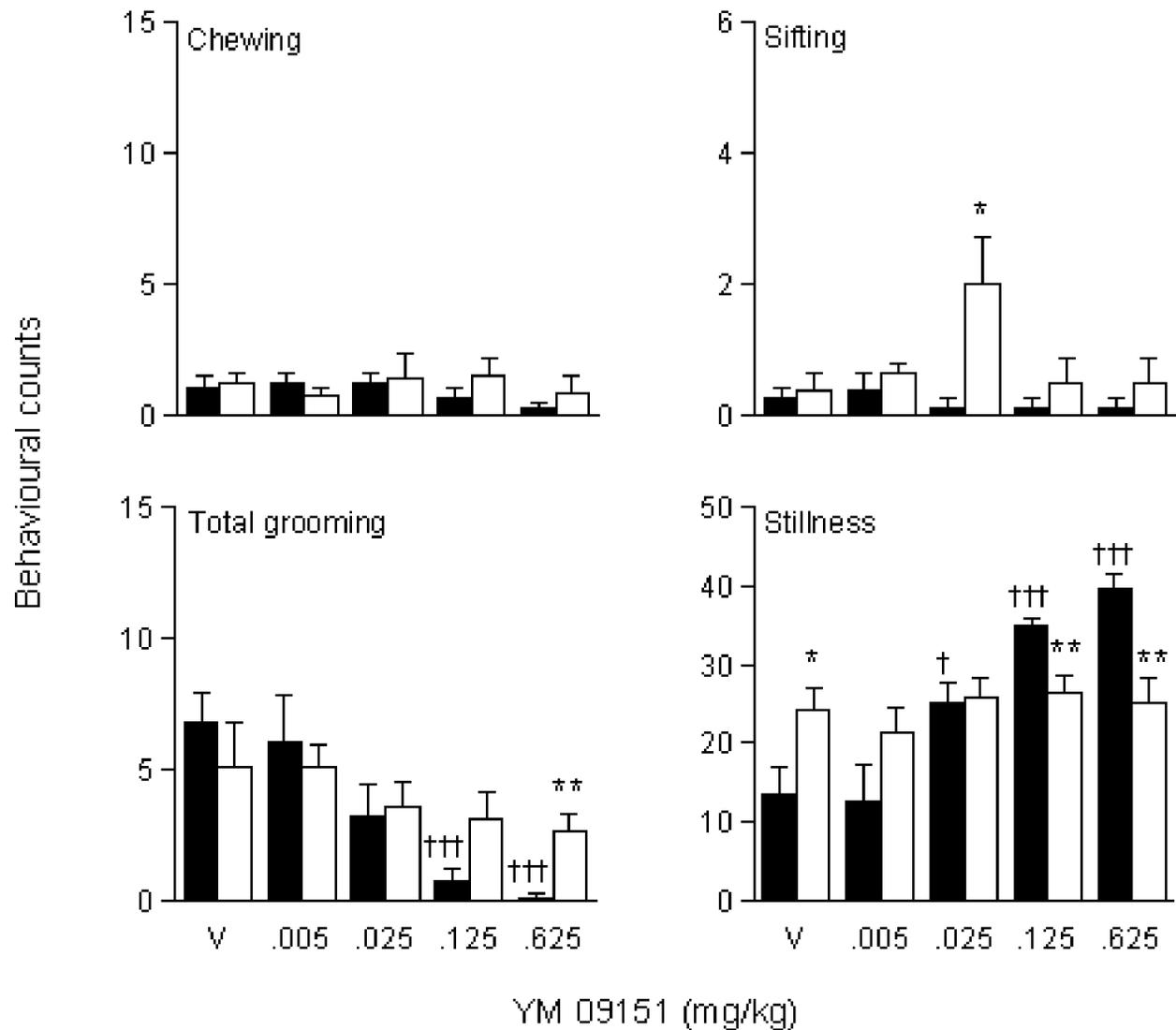


Figure 4