

Receptor Crosstalk: Characterization of Mice Deficient in Dopamine D₁ and Adenosine A_{2A} Receptors

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Here we report the development of D₁A_{2A} receptor knockout mice to investigate whether interactions between dopamine D₁ and adenosine A_{2A} receptors participate in reward-related behavior. The combined deletion of D₁ and A_{2A} receptors resulted in mice with decreased weight and appetitive processes, reduced rearing and exploratory behaviors, increased anxiety, and a significantly poorer performance on the rotarod, compared to wild-type littermates. D₁A_{2A} receptor knockout mice shared phenotypic similarities with mice deficient in D₁ receptors, while also paralleling behavioral deficits seen in A_{2A} receptor knockout mice, indicating individual components of the behavioral phenotype of the D₁A_{2A} receptor knockout attributable to the loss of both receptors. In contrast, ethanol and saccharin preference in D₁A_{2A} receptor knockout mice were distinctly different from that observed in derivative D₁ or A_{2A} receptor-deficient mice. Compared to wild types, preference and consumption of ethanol were decreased in D₁A_{2A} receptor knockout mice, the reduction in ethanol consumption greater even than that seen in D₁ receptor-deficient mice. Preference and consumption of saccharin were also reduced in D₁A_{2A} receptor knockout mice, whereas saccharin preference was similar in wild-type, D₁, and A_{2A} receptor knockout mice. These data suggest an interaction of D₁ and A_{2A} receptors in the reinforcement processes underlying the intake of rewarding substances, whereby the A_{2A} receptor seems involved in goal-directed behavior and the motor functions underlying the expression of such behaviors, and the D₁ receptor is confirmed as essential in mediating motivational processes related to the repeated intake of novel substances and drugs.

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INTRODUCTION

The mesolimbic pathway has been heavily implicated in the neuronal processes underlying motivational responses to reinforcing substances or rewarding stimuli (Wise, 1996), with the dopaminergic projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) implicated in both movement and reward-related function (Self and Nestler, 1995; Koob, 1999). Recently, however, it has been suggested that the role of dopamine is more precisely that of mediating goal-directed behaviors related to reward (Cannon and Palmiter, 2003), or that dopamine neurotransmission is involved in processing the 'unexpectedness' of an event, rather than representing the rewarding 'value' of the stimulus (Schultz *et al*, 1997).

Dopamine and adenosine receptors share an extensive codistribution within forebrain regions implicated in motivational and motor processes, and there is much evidence that adenosine A_{2A} receptor activation is able to influence dopaminergic function (and effects mediated via D₁ and D₂ receptors) and vice versa (Ferré *et al*, 1997). GABAergic medium spiny neurons of the dorsal striatum have been divided into two populations—the striato-nigral/entopeduncular (substance P, dynorphin-expressing) and striato-pallidal (enkephalin-expressing) efferents (eg Gerfen *et al*, 1990; Kawaguchi *et al*, 1990; Reiner and Anderson, 1990; Le Moine *et al*, 1991; Steiner and Gerfen, 1993). Dopamine D₁ and D₂ receptors are, for the most part, distributed on separate neuronal populations (eg Le Moine and Bloch, 1995), with D₂ and A_{2A} receptors (in the main) colocalized upon the same neuronal population, and D₁ and A_{2A} receptors largely segregated (Svenningsson *et al*, 1997, 1999). The adenosine A_{2A} receptor is therefore considered an indirect target by which to modulate dopamine-related functions (Ferré *et al*, 1997).

Strategies to investigate the role of the A_{2A} receptor in basal ganglia function have included the genetic approach of double receptor deletion, such as D₂A_{2A} and CB₁A_{2A}

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receptor knockout mice (Chen *et al*, 2001; Berrendero *et al*, 2003). While the phenotype of the D₂A_{2A} receptor knockout remains to be fully characterized (Chen *et al*, 2001), potential interactions between CB₁ and A_{2A} receptors, at least with regards to opiate withdrawal, were not established (Berrendero *et al*, 2003). Unlike D₂ and A_{2A} receptor interactions, interactions involving D₁ and A_{2A} receptors are contingent upon a degree of 'crosstalk' or interactions occurring at a network level, as D₁ and A_{2A} receptors are segregated upon distinct neuronal populations. The combined deletion of D₁ and A_{2A} receptors was thus considered an appropriate approach to examine the interaction between D₁ and A_{2A} receptor systems, as the phenotype of wild-type, D₁, A_{2A}, and D₁A_{2A} receptor knockout mice could be directly compared. The D₁A_{2A} receptor knockout mouse characterized in the current study was then used to investigate whether dopamine-adenosine receptor interactions influence striatal neurochemistry or underpin complex behaviors (such as ethanol and saccharin preference).

MATERIALS AND METHODS

Experimental Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986, under the guidelines of the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. All experiments were completed on adult mice. A_{2A} receptor knockout mice developed on a CD-1 background (Ledent *et al*, 1997) were backcrossed with C57/Bl6J mice for four generations, to enable breeding with D₁ receptor knockout mice that had been previously developed on a C57Bl/6J background (Drago *et al*, 1994). To overcome potential strain effects, appropriate wild-type animals were obtained from litters generated by heterozygous breeders. Equal numbers of male and female mice from each genotype were assessed for all behavioral experiments. The genotype of experimental mice was confirmed as previously described (Drago *et al*, 1994; Snell *et al*, 2000). Mice received laboratory chow and water *ad libitum*, and were kept in a constant 12-h light-dark cycle (light 0700–1900 hours).

Materials

Oligonucleotide probes were synthesized by Auspep Pty Ltd (Parkville, Victoria, Australia). [α -³³P]-dATP, [³H]-CGS-21680, [³H]-mazindol, [¹²⁵I]-SCH 23982 were purchased from PerkinElmer Life Sciences (Boston, MA). Na[¹²⁵I] was from Amersham Biosciences UK, Ltd (Little Chalfont, Buckinghamshire, UK). DMPA and GBR 12935 were purchased from Research Biochemicals International (Sigma-Aldrich; Missouri, MO), and SKF 77434 was from Sigma/RBI (Natick, MA). NCQ 634 and raclopride were both gifts from Astra (Hässel, Mölndal, Sweden). All other chemicals and reagents were either laboratory or analytical grade and purchased from various sources.

In Vivo Experiments

Two experimenters, blind to the genotype of the mouse, performed all behavioral tests, with each observation

recorded in duplicate and later correlated. All tests were completed in the same room with consistent lighting conditions (nondirect illumination, 60 Lux), and mice were allowed to adjust to the environment before testing began. The general health and abilities of all mice were examined with a modified Irwin screen to determine the presence of gross alterations in appearance or behavior (for the full experimental protocol, see Irwin, 1968). All mice were used only once and were naïve to the behavioral paradigm, with a minimum of $N=8$ for each test.

Open field. The open field consisted of an area 40 cm by 50 cm, enclosed by 9 cm high walls. Mice were placed in the center of the open arena, facing away from experimenters. The test session was 5 min and began immediately. Time taken for the mouse to make contact with any wall was recorded, with the wall-seeking latency used as an indicator of anxiety. Within the 5-min period, the number of rears (two front paws off floor), both open and against wall, were recorded.

The holeboard apparatus. The holeboard design was a square platform (40 cm²) enclosed by four walls (15 cm high) and raised 10 cm off the floor. In the base of the platform four holes of equal diameter (3 cm) were cut, each an equal distance from the walls. The mouse was placed in the center of the base, and the number of full head dips (through to the shoulder) was counted over a 1-min period. This test was considered an assay of exploration.

The elevated plus maze. The elevated plus maze was a right-angled cross design, and the task completed as described elsewhere (Ross *et al*, 2000). Anxiety levels were correlated with time spent on open arms as a percentage of trial time, and the total number of open and closed transitions was used as a measure of locomotor activity (number of entries). An open or closed arm entry was defined as all four paws leaving the central square.

The rotarod apparatus. The mouse was placed on the stationary rotarod (Ugo Basile, Italy) facing away from the experimenter. Timing was initiated as the rotarod accelerated (4–40 r.p.m. over 5 min). The latency of the mouse to fall off the rotarod was recorded over four consecutive trials.

The single beam and parallel strings tests. The single beam apparatus was a length of wood (40 cm long by 3 cm wide) attached to two platforms at either end (4 cm by 7 cm and 7 cm above the floor). Mice were placed on the beam at the end closer the experimenter and time taken to reach the other platform was recorded. For the parallel strings apparatus, two strings (30 cm in length; diameter 2 mm) were attached across opposing walls of an open box (30 cm by 45 and 15.5 cm deep) 2 cm apart. Mice were placed on the strings at the origin and the time taken to cross to the opposite wall was recorded. Both tests were halted after 2 min, and if the mouse had not completed the task, distance traveled (in cm) was measured.

Consumptive Behaviors

Experimental mice were housed individually and placed on a 28-day continual-access free-choice drinking program to allow the determination of ethanol or saccharin consumption. Mice were assigned to one of two experimental conditions: receiving a choice between two bottles containing either a 5% (v/v) ethanol solution or water; or receiving a choice between two bottles containing either a 0.1% (w/v) saccharin solution or water ($N=8$ per experimental condition). In a previous study in dopamine D₁ receptor knockout mice, free-choice ethanol preference was investigated using a limited access paradigm at concentrations of 3, 6, and 12%, and also 12% ethanol using a continual access paradigm. At all concentrations examined, ethanol preference and intake in D₁ receptor knockout mice were reduced by a similar factor, compared to wild-type mice (El-Ghundi *et al*, 1998). We have therefore chosen an appropriate concentration of ethanol; in the middle of the range examined by El-Ghundi *et al* (1998), where palatability and preference are high, allowing reliable quantification of data. Standard wire cage lids were adjusted to accommodate the two bottles, with equal access to both stoppers. These bottles were exchanged in a random manner to prevent place preference and the fluid consumed was recorded at a consistent time daily. The average consumption of water, the test solution (ethanol or saccharin), and the total fluid intake were calculated (ml, per kg body weight, per day). The average dose of each test solution consumed (g or mg, per kg body weight, per day) and the relative preference for a particular test solution (ml test solution as a ratio of the total daily fluid intake) were also determined.

In Vitro Experiments

Mice were killed via cervical dislocation followed by rapid decapitation. Whole mouse brains were frozen over liquid nitrogen and stored at -80°C until further processing. Cryostat (Cryocut 1800; Leica, Wetzlar, Germany) cut coronal sections (14 μm) were collected through the NAcc, ventral pallidum (VP), and VTA, with the appropriate anatomic levels determined according to the atlas of Franklin and Paxinos (1997).

In situ hybridization histochemistry. The experimental procedures described herein were developed from previous studies (McLean *et al*, 1996; Chen *et al*, 1998). Oligonucleotide probes were 3'-end labelled with [α - ^{32}P]-dATP, to a specific activity of between 1.0 and 3.0×10^5 d.p.m./ μl . Nonspecific hybridization was determined in the presence of a 100-fold excess of unlabelled oligonucleotide (relative to the molar concentration of the labelled probe). Oligonucleotide probe sequences are as follows: dopamine D₁ receptor mRNA (Drago *et al*, 1994); dopamine D₂ receptor mRNA (Le Moine *et al*, 1990); adenosine A_{2A} receptor mRNA (Ledent *et al*, 1997); dopamine transporter (DAT) mRNA: 5'-AGTTATTGGT-GAACTTATTG-TAACTGGAGA-AGGCAATCAG-CAC-3'; preproenkephalin mRNA: 5'-ATCTGCATCC-TTCTTCATGA-AGCCGCCATA-CCTCTTGGCA-AGGATCTC-3'.

Receptor and transporter autoradiography. D₁ receptor autoradiography using [^{125}I]-SCH 23982 (0.01 nM) was completed following a published protocol (Djouma and Lawrence, 2002). Nonspecific binding was defined as that remaining in the presence of (\pm)-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SKF 77434) (10 μM). *N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide (NCQ 634) was iodinated to (S)-3-iodo-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-5,6-dimethoxysalicylamide ([^{125}I]-NCQ 298) with this procedure and the methodology for D₂-dopamine receptor autoradiography described elsewhere (Lawrence *et al*, 1995). Binding remaining in the presence of raclopride (10 μM) was defined as nonspecific. A_{2A} receptor autoradiography was completed essentially as previously described, with [^3H]-CGS 21680 (5 nM) (Johansson and Fredholm, 1995). Nonspecific binding was defined as that remaining in the presence of *N*⁶-[2-(3,5-dimethoxyphenyl)-2-(methylphenyl)ethyl]adenosine (DPMA; 10 μM). DAT autoradiography using [^3H]-mazindol (4 nM) was completed according to published protocols (Donnan *et al*, 1989). Nonspecific binding was that remaining in the presence of 1-(2-[diphenylmethoxy]ethyl)-4-[3-phenylpropyl]piperazine dihydrochloride (GBR 12935) (20 μM).

Development and quantification. [^{33}P]- and [^{125}I]-labelled sections were apposed to X-ray film (Eastman Kodak; Rochester, NY) in the presence of standard ^{14}C microscales (American Radiolabelled Chemicals, Inc.; St Louis, MO), and [^3H]-labelled sections were apposed to tritium-sensitive Hyperfilm- ^3H in the presence of ^3H microscales (film and microscales; Amersham International, UK). Optical densities were converted to radioactivity per unit area and presented as d.p.m./mm², using an MCID (micro computing imaging device) M4 analysis system (Imaging Research, St Catharines, Ontario, Canada). For internal consistency all slides in a particular hybridization or autoradiography experiment were processed, apposed to film, developed, and analyzed concurrently.

Statistics

Data are presented as the mean \pm SEM, and $p < 0.05$ was considered significant. For the statistical analysis of behavioral data, three-way ANOVA were performed, with the three factors being D₁ receptor deletion/presence, A_{2A} receptor deletion/presence, and gender. This statistical design allowed the interaction between D₁ and A_{2A} receptors to be investigated. When a main effect of gender was determined, male and females were analyzed separately using a two-way ANOVA design. If there was no main effect of gender, and no interactions between factors, two-way or one-way ANOVA was performed, with all ANOVAs followed by Holm-Sidak and *t*-test *post hoc* analyses, to assess the influence of D₁ and A_{2A} receptor deletion (and gender where appropriate) upon phenotype. To facilitate discussion of the results, interactions between factors are reported only when significant; thus where $p > 0.05$, results of three- and two-way ANOVA are not included. As only male mice were used in the neurochemical studies, autoradiography and hybridization densities were analyzed using two-way and one-way ANOVA for multiple comparisons, or a *t*-test as appropriate. ANOVA were followed by a *post hoc*

Dunnett's *t*-test. If the data failed tests for normality or homogeneity of variance, nonparametric tests were employed. In these cases, data were then analyzed with either a Mann–Whitney rank sum test or a Kruskal–Wallis one-way ANOVA by ranks, followed by a *post hoc* Dunn's *t*-test. A Bonferroni correction (*p* divided by the number of statistical tests in a given experiment) was completed to overcome the effect of multiple comparisons.

RESULTS

D₁A_{2A} receptor knockout mice survived well into adulthood if certain precautions were taken, such as late weaning (to physically strengthen the mouse and allow the mother to wean the pups herself and 'encourage' their eating habits), separation of litters of wild-type and mutant mice, and the availability of an alternative palatable food source (rodent chow dust mixed with peanut butter). Statistical analysis determined a main effect of gender on weight ($F_{1,36} = 49.53$, $p < 0.001$), with male mice weighing more than female mice, and therefore the genders were separated and male and female mice analyzed further in a two-way ANOVA. For female mice, a main effect of D₁ receptor deletion/presence ($F_{1,36} = 110.80$, $p < 0.001$) and an interaction between D₁ and A_{2A} receptor deletion/presence on weight was detected ($F_{1,36} = 17.16$, $p < 0.001$). *Post hoc* analyses of body weight revealed that adult female A_{2A}, D₁, and D₁A_{2A} receptor knockout mice were significantly lighter in weight than wild-type mice (Figure 1a). In addition, female D₁ and D₁A_{2A} receptor knockout mice were significantly lighter in weight than A_{2A} receptor knockout mice, with no further weight differences between D₁ and D₁A_{2A} receptor knockout mice (Figure 1a). For male mice, a main effect of D₁ receptor deletion/presence ($F_{1,36} = 83.87$, $p < 0.001$) was detected, with further analyses determining that adult male D₁A_{2A} and D₁ receptor knockout mice were significantly lighter in weight than wild-type and A_{2A} receptor knockout mice, with no weight differences between D₁ and D₁A_{2A} receptor knockout mice or wild-type and A_{2A} receptor knockout mice (Figure 1b).

In Vivo Experiments; Behavioral Characterization of D₁A_{2A} Receptor Knockout Mice

The general health and abilities of all mice were examined, with no evidence of lacrimation, salivation, gross disturbances in respiration, altered skin color, tremors, or convulsions observed. Mice were active and exhibited a 'normal' coat appearance (fur in good condition, well groomed), normal movement, and gait. Defecation and urination rates were normal, and the visual placing reflex was present in all mice.

Within the open arena test, no differences in the latency of mice to move to a position against an arena wall were determined (data not shown). A main effect of the deletion/presence of the D₁ receptor on the number of rearing episodes was detected ($F_{1,36} = 38.32$, $p < 0.001$), with *post hoc* analyses revealing both D₁ and D₁A_{2A} receptor knockout mice exhibited a significantly reduced number of rearing episodes, compared to wild-type and A_{2A} receptor knockout mice (Figure 1c). A main effect of the deletion/presence of

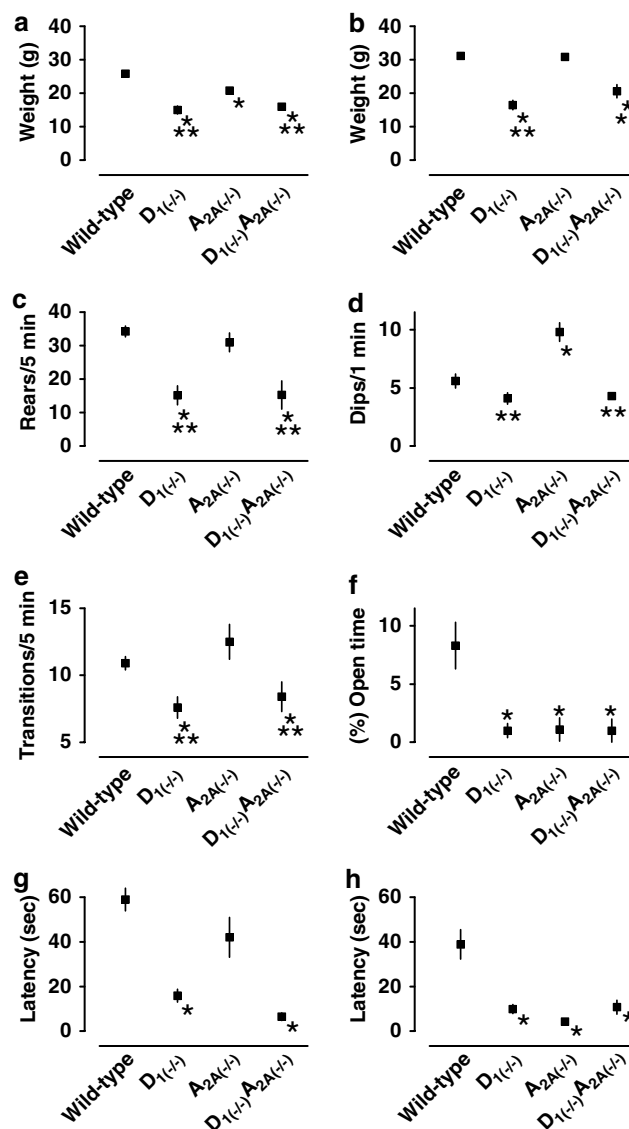


Figure 1 Behavioral comparisons between wild-type and D₁, A_{2A} and D₁A_{2A} receptor knockout mice. Adult body weight (g) of female (a) and male (b) mice. The number of rearing episodes in an open arena 5 min trial (c). The total number of holeboard explorations over 1 min; defined as head-dips to the shoulder region (d). The total number of elevated plus maze transitions (closed plus open) (e). The time spent in the open arms of the elevated plus maze, as a percentage of the overall test period (5 min) (f). Mean fall latencies measured over four rotarod trials of female (g) and male (h) mice. Significant differences compared to wild-type (* $p < 0.05$), significant differences compared to A_{2A} receptor knockout mice (** $p < 0.05$); three-way ANOVA, followed by two-way or one-way ANOVA and *post hoc* tests where appropriate; $N = 8$, per genotype examined.

the D₁ receptor ($F_{1,36} = 37.31$, $p < 0.001$) and deletion/presence of the A_{2A} receptor ($F_{1,36} = 13.93$, $p < 0.001$) on the number of holeboard explorations was detected, with *post hoc* analyses revealing that the number of holeboard explorations was significantly increased in A_{2A} receptor knockout mice, compared to wild-type, D₁ and D₁A_{2A} receptor knockout mice (Figure 1d). Small reductions in the number of holeboard explorations performed by D₁ and D₁A_{2A} receptor knockout mice were not significantly

different when compared to wild-type mice, but in the D₁A_{2A} receptor knockout the additional loss of the D₁ receptor effectively resulted in the loss of the more explorative phenotype exhibited by mice deficient in A_{2A} receptors (Figure 1d). Upon the elevated plus maze, the total number of transitions was significantly reduced in mice lacking D₁ and D₁A_{2A} receptors, compared to wild-type and A_{2A} receptor knockout mice (main effect of D₁ receptor deletion/presence; $F_{1,36} = 20.69$, $p < 0.001$ (Figure 1e)). In addition, a main effect of D₁ receptor deletion/presence ($F_{1,36} = 6.53$, $p < 0.05$) and A_{2A} receptor deletion/presence ($F_{1,36} = 6.00$, $p < 0.05$), and an interaction between D₁ and A_{2A} receptor deletion/presence ($F_{1,36} = 6.25$, $p < 0.05$) on the proportion of time spent in the open arms of the elevated plus maze were detected. After collapsing the data across gender and performing a two-way ANOVA, the interaction between factors remained (D₁ and A_{2A} receptor deletion/presence), with *post hoc* analyses revealing time spent on the open arm to be significantly reduced in all mutant mice examined, compared to wild types (Figure 1f). Finally, motor coordination or performance on the rotarod apparatus was significantly impaired in D₁, A_{2A}, and D₁A_{2A} receptor knockout mice, as indicated by the significantly reduced fall latencies (main effect of D₁ receptor deletion/presence ($F_{1,36} = 51.41$, $p < 0.001$), A_{2A} receptor deletion/presence ($F_{1,36} = 18.06$, $p < 0.001$), and an interaction between D₁ and A_{2A} receptor deletion/presence ($F_{1,36} = 9.25$, $p < 0.01$) on rotarod fall latencies). A main effect of gender on rotarod fall latencies was also observed ($F_{1,36} = 17.85$, $p < 0.001$), and therefore female and male mice were separated and analyzed using a two-way ANOVA. For female mice, a main effect of D₁ receptor deletion/presence ($F_{1,36} = 48.30$, $p < 0.001$) and A_{2A} receptor deletion/presence ($F_{1,36} = 5.39$, $p < 0.05$) was detected, with further analyses determining that female D₁ and D₁A_{2A} receptor knockout mice exhibited a decreased latency to fall on the rotarod apparatus, compared to wild-type mice (Figure 1g). For male mice, a main effect of D₁ receptor deletion/presence ($F_{1,36} = 7.38$, $p < 0.05$), A_{2A} receptor deletion/presence ($F_{1,36} = 16.52$, $p < 0.001$), and an interaction between D₁ and A_{2A} receptor deletion/presence on rotarod performance was detected ($F_{1,36} = 18.36$, $p < 0.001$). *Post hoc* analyses revealed that male A_{2A}, D₁, and D₁A_{2A} receptor knockout mice performed poorly on the rotarod apparatus, exhibiting a reduced latency to fall compared with wild-type mice (Figure 1h). Interestingly, D₁ and D₁A_{2A} receptor-deficient mice were also unable to perform tasks designed to investigate motivation and motor properties such as balance and coordination. Thus, D₁ and D₁A_{2A} receptor knockout mice would not traverse a single suspended beam or two parallel strings. Conversely, all wild-type mice tested were able to complete these tasks successfully (data not shown).

Consummatory Behavioral Data

A main effect of D₁ receptor deletion/presence ($F_{1,31} = 10.89$, $p < 0.01$) on preference for the ethanol solution was detected, with no main effects of A_{2A} receptor deletion/presence, gender, or interactions between factors. Further analyses showed that D₁ and D₁A_{2A} receptor knockout mice exhibited a reduced preference for the ethanol solution,

when compared with wild-type mice (Figure 2b). A main effect of D₁ receptor deletion/presence ($F_{1,31} = 15.97$, $p < 0.001$) and A_{2A} receptor deletion/presence ($F_{1,31} = 8.49$, $p < 0.01$) on ethanol consumption was also observed, and *post hoc* tests revealed that D₁ and D₁A_{2A} receptor knockout mice ingested significantly less ethanol (5% v/v)

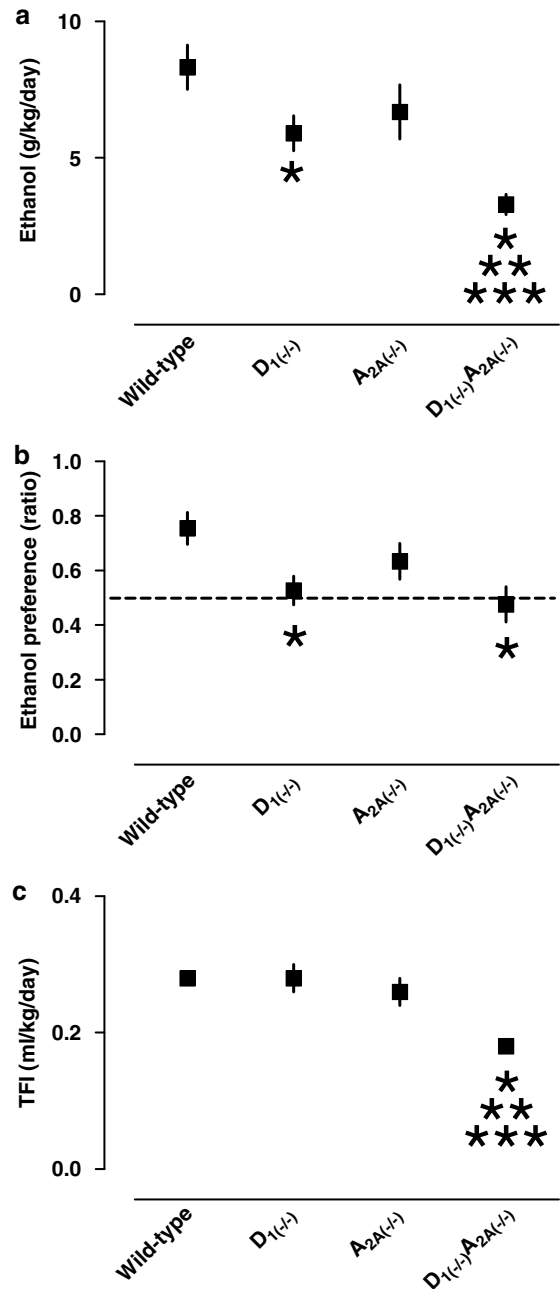


Figure 2 Consummatory behavior in wild-type and D₁, A_{2A}, and D₁A_{2A} receptor knockout mice. Daily consumption of ethanol (5% v/v), as calculated using a 28-day free-choice behavioral paradigm in wild-type and mutant mice (a). Preference for the ethanol solution over water; calculated by the ratio of daily ethanol consumption to total daily fluid intake (b). Total daily fluid intake (TFI) (ethanol plus water consumption) (c). Significant differences compared to wild-type (* $p < 0.05$), significant differences compared to A_{2A} receptor knockout mice (** $p < 0.05$), significant differences compared to D₁ receptor knockout mice (*** $p < 0.05$); three-way ANOVA, followed by two-way or one-way ANOVA and *post hoc* tests where appropriate; $N = 8$, per genotype examined.

than wild-type controls (Figure 2a). In addition, a main effect of D₁ receptor deletion/presence ($F_{1,31}=5.26$, $p<0.05$), A_{2A} receptor deletion/presence ($F_{1,31}=15.86$, $p<0.001$), and an interaction between D₁ and A_{2A} receptor deletion/presence ($F_{1,31}=7.19$, $p<0.05$) on total fluid consumption (ethanol plus water) was observed. *Post hoc* analyses showed that D₁A_{2A} receptor knockout mice ingested less ethanol (g/kg per day) and fluid in total than mice deficient in either D₁ or A_{2A} receptors, but as water consumption was increased in D₁ receptor knockout mice (a main effect of D₁ receptor deletion/presence ($F_{1,31}=6.51$, $p<0.05$) on water intake), preference for the ethanol solution over water was similar in D₁ and D₁A_{2A} receptor knockout mice (Figure 2a–c). Indeed, in D₁ and D₁A_{2A} receptor knockout mice, the preference ratio was effectively 0.5, that is, neither preference nor aversion exhibited to the ethanol solution.

A main effect of D₁ receptor deletion/presence ($F_{1,31}=19.27$, $p<0.001$), and an interaction between D₁ and A_{2A} receptor deletion/presence ($F_{1,31}=7.78$, $p<0.01$) on saccharin preference was detected. In addition, a main effect of D₁ receptor deletion/presence ($F_{1,31}=68.37$, $p<0.001$) and A_{2A} receptor deletion/presence ($F_{1,31}=9.20$, $p<0.01$) on daily saccharin consumption was observed, with no interactions between factors. *Post hoc* analyses revealed that while preference for the saccharin (0.1% w/v) solution was not altered in D₁ receptor knockout mice compared to wild-type mice, D₁ receptor knockout mice demonstrated a reduced daily saccharin consumption rate (Figure 3a and b). Conversely, in D₁A_{2A} receptor mutant mice, saccharin preference and daily saccharin consumption were reduced from that observed in wild-type mice, and comparatively, preference for the saccharin solution over water was significantly lower in D₁A_{2A} knockout mice than that exhibited by mice with the single deletion of D₁ or A_{2A} receptors (Figure 3a and b). In addition, a main effect of D₁ receptor deletion/presence ($F_{1,31}=89.82$, $p<0.001$) and A_{2A} receptor deletion/presence ($F_{1,31}=14.74$, $p<0.001$) on total fluid intake (saccharin plus water) was observed, with *post hoc* analyses revealing a lower total fluid intake in A_{2A}, D₁, and D₁A_{2A} receptor knockout mice, compared to wild-type (Figure 3c). While water intake was not significantly different between the genotypes of mice examined (data not shown), consumption of the saccharin solution was substantially higher in wild-type mice, resulting in the observed differences in total fluid intake and indeed the higher overall total fluid intake in wild-type mice for the saccharin trial.

In Situ Hybridization Histochemistry

D₁ receptor mRNA was quantified in the caudate-putamen (CPu), NAcc, and olfactory tubercle (OT) of wild-type mice, while expression of D₁ receptor mRNA was not observed in D₁A_{2A} receptor-deficient mice. The oligonucleotide sequence was complementary to a section of D₁ receptor mRNA deleted in the D₁ receptor knockout mouse, hence the absence of signal in these mutants. D₂ receptor mRNA was quantified in the CPu, NAcc, and OT, and also the substantia nigra pars compacta (SNc) and VTA, both in wild-type and receptor knockout mice. No significant

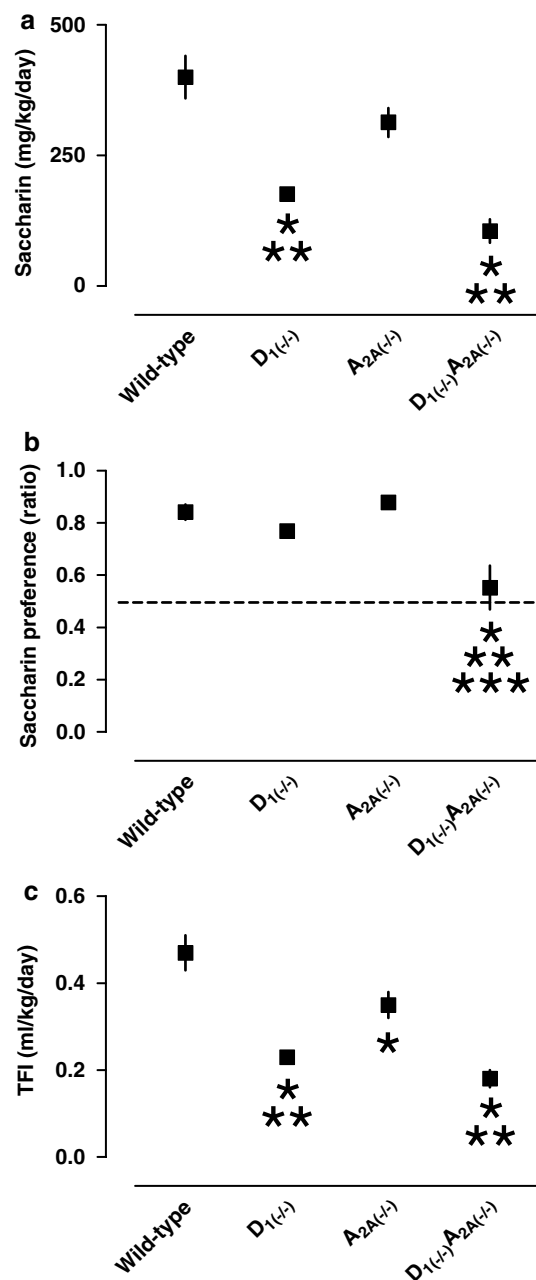


Figure 3 Consumatory behavior in wild-type and D₁, A_{2A} and D₁A_{2A} receptor knockout mice. Daily consumption of saccharin (0.1% w/v), as calculated using a 28-day free-choice behavioral paradigm in wild-type and mutant mice (a). Preference for the saccharin solution over water; calculated by the ratio of daily saccharin consumption to total daily fluid intake (b). Total daily fluid intake (TFI) (saccharin plus water consumption) (c). Significant differences compared to wild-type (* $p<0.05$), significant differences compared to A_{2A} receptor knockout mice (** $p<0.05$), significant differences compared to D₁ receptor knockout mice (*** $p<0.05$); three-way ANOVA, followed by two-way or one-way ANOVA and *post hoc* tests where appropriate; $N=8$, per genotype examined.

differences in D₂ receptor transcript between mutant and control mice were detected (data not included). A_{2A} receptor mRNA hybridization was detected in the CPu, NAcc, and OT of wild-type mice, while the hybridization signal in D₁A_{2A} receptor knockout mice resembled that of

nonspecific background. This confirms the absence of the A_{2A} receptor transcript for which the A_{2A} receptor mRNA oligonucleotide was directed; similar to the D₁ receptor oligonucleotide, the A_{2A} receptor mRNA probe was directed to a section of the sequence deleted in A_{2A} receptor mutant mice. DAT mRNA was observed in wild-type and double receptor knockout mice and was restricted to the SNc and VTA. No differences in DAT mRNA were detected between D₁A_{2A} and wild-type mice (data not shown). Preproenkephalin mRNA detected in the CPu, NAcc, and OT of wild-type and D₁A_{2A} receptor-deficient mice was unchanged in D₁A_{2A} receptor knockout mice, compared to wild types (data not shown).

Autoradiography

D₁ receptor binding was observed in the CPu, NAcc, OT, and VP, and within the substantia nigra pars reticulata (SNr) using [¹²⁵I]-SCH 23982. No D₁ receptor binding was detected in mice deficient in D₁A_{2A} receptors; in these mice [¹²⁵I]-SCH 23982 binding was indistinguishable from nonspecific binding seen in wild-type brain slices. D₂ receptors were examined using [¹²⁵I]-NCQ 298, and dense binding was observed in wild-type and D₁A_{2A} receptor knockout mice (CPu, NAcc, OT, SNr, SNc, VTA). Significant alterations in double receptor knockout mice were restricted to a small (13 ± 3%) increase in [¹²⁵I]-NCQ 298 binding in the CPu of D₁A_{2A} receptor-deficient mice, compared to wild types (nonsignificant data not included). Binding of [³H]-CGS 21680 was observed in the CPu, NAcc, OT, and VP in wild-type mice, and was entirely absent in mice deficient in functional D₁A_{2A} receptors. DAT binding was visualized using [³H]-mazindol, and detected in forebrain regions such as the CPu, NAcc (separated into lateral, medial, and rostral pole on the basis of differential regional binding profiles), and OT, and also quantified in the SNc, SNr, and VTA within the ventral mesencephalon. D₁A_{2A} receptor knockout mice were compared to their wild-type controls, and in mice deficient in D₁A_{2A} receptors significant (but small) alterations in [³H]-mazindol binding were observed, that is, a 17 ± 4 and 12 ± 2% reduction in the density of the DAT in the rostral pole of the NAcc and SNr, respectively, of D₁A_{2A} receptor knockout mice compared to wild-types.

To investigate the influence of dopamine or adenosine receptor deletion on the corresponding receptor expression, further experiments were completed on derivative D₁ and A_{2A} receptor knockout mice. A_{2A} receptor mRNA expression was increased in the CPu of D₁ receptor knockout mice (Figure 4d). Large increases in A_{2A} receptor density, as determined using [³H]-CGS 21680, were also found in D₁ receptor-deficient mice, compared to wild-type mice (Figure 4h). D₁ receptor mRNA was also found to be significantly increased in the CPu and NAcc of A_{2A} receptor knockout mice, compared with wild-types (Table 1).

DISCUSSION

Mice deficient in functional D₁ and A_{2A} receptors (D₁A_{2A} receptor knockout mice) were successfully developed, and

found to exhibit a reduced preference and consumption of ethanol and saccharin, compared to wild-type. These findings suggest an interaction between D₁ and A_{2A} receptors pertinent to goal-directed behavior, an hypothesis supported by the upregulation of striatal A_{2A} receptors in D₁ receptor-deficient mice.

Like the D₁ receptor knockout (Drago *et al*, 1994), D₁A_{2A} receptor knockout mice exhibited reduced body weight, lower rearing frequency in the open arena, made less total transitions and spent less time on the open arms of the elevated plus maze, and performed significantly worse on the rotarod apparatus, compared to wild-type mice. Overall, therefore, loss of the A_{2A} receptor had no measurable ability to improve the phenotypic impairments induced by D₁ receptor deletion, at least for the current behavioral assessments. In addition, there was no significant worsening of the general behavioral phenotype exhibited by the D₁A_{2A} receptor knockout as compared to the D₁ and A_{2A} receptor-deficient mice in the present study. For example, each of these mutants appeared anxious on the elevated plus maze, as indicated by a significant reduction in the proportion of time spent on the open arm; however, as the phenotype exhibited by all three genotypes was of substantial open arm avoidance, a ceiling effect may have been confounding. Moreover, other behavioral measures were poorly performed in mice with the genetic ablation of the D₁ receptor (ie rotarod) and thus the ability to detect further impairments in the D₁A_{2A} receptor knockout may have been compromised. Conversely, the lack of a 'recovered' phenotype in D₁A_{2A} receptor-deficient mice may simply be due to adenosine primarily acting as a neuromodulator. As such, adenosine may regulate dopaminergic activity but not directly mediate the aspects of anxiety, motor, and exploratory behaviors examined. Furthermore, compensatory adaptations remain a possibility, as these mice have been receptor ablated throughout development.

D₁ and D₁A_{2A} receptor knockout mice consumed less ethanol each day and exhibited a reduced preference for the 5% v/v ethanol solution than wild-type mice. Reduced ethanol consumption and preference, irrespective of the concentration of ethanol provided, was also reported in an earlier study examining D₁ receptor knockout mice (El-Ghundi *et al*, 1998). A phenotype of reduced ethanol intake and preference in mice with the genetic deletion of the D₁ receptor are difficult to reconcile with the concept proposing reward processing and goal-directed behaviors are mediated by distinct neuronal systems (Cannon and Palmiter, 2003), as a reduced ethanol consumption would be expected, with no change in preference. Schultz (2002) has suggested a separation of dopamine function by availability and release characteristics, whereby tonic dopamine in the striatum acting upon the high-affinity D₂ receptor is associated with motor coordination and goal-directed behaviors, while phasic dopamine release activating lower affinity D₁ receptors mediates the unexpectedness of an event. Therefore, mice with the genetic deletion of the D₁ receptor may not have the receptor architecture for the initial phasic release of dopamine that identifies a 'rewarding' event has been experienced; hence, ethanol preference is not established. In support, an examination of water intake in D₁ receptor knockout mice reveals that the reduced preference for ethanol has resulted in the reduced

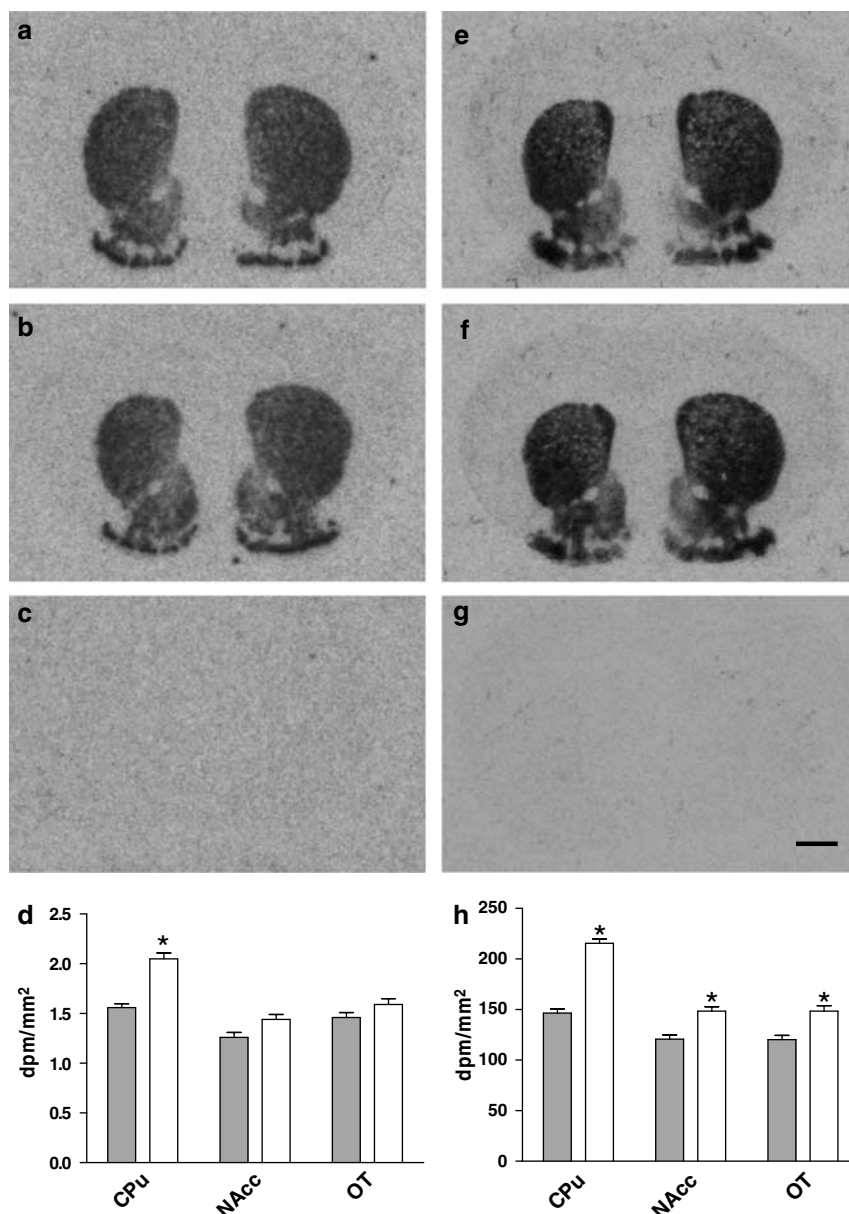


Figure 4 Representative autoradiograms showing *in situ* hybridization histochemistry images of A_{2A} receptor transcript on coronal sections (12 μm) from wild-type (a), D₁ receptor knockout (b) and A_{2A} receptor knockout (c) mice. A_{2A} receptor mRNA hybridization was quantified in the caudate-putamen, nucleus accumbens, and olfactory tubercle in the forebrain of wild-type (hatched bar) and D₁ receptor knockout mice (white bar) (d). Representative autoradiograms demonstrating [³H]-CGS 21680 (5 nM) binding to A_{2A} receptors on coronal sections (12 μm) from wild-type (e), D₁ receptor knockout (f) and A_{2A} receptor knockout (g) mice. Specific [³H]-CGS 21680 binding was quantified in the caudate-putamen, nucleus accumbens, and olfactory tubercle of wild-type (hatched bar) and D₁ receptor knockout mice (white bar) (h). The scale bar represents 1.0 mm. Key: CPU, caudate-putamen; NAcc, nucleus accumbens; OT, olfactory tubercle. Significant differences (**p* < 0.05); *t*-test or Mann–Whitney rank sum test. *N* = 4–6 mice per genotype (12 sections per mouse).

ethanol consumption observed in these mutants, as overall total daily fluid intake is no different to wild-type mice.

The ethanol nonpreferring phenotype of D₁A_{2A} receptor knockout mice was distinct from that observed in D₁ receptor knockout mice. D₁A_{2A} receptor knockout mice exhibit reduced preference for 5% v/v ethanol, combined with a significantly reduced total daily fluid intake, suggesting that under the conditions of our experiment, mechanisms underlying both the reinforcement of ethanol preference, and goal-directed behavior to consume ethanol

appear impaired. Ethanol intake in D₁A_{2A} receptor-deficient mice was reduced by over 40% compared to D₁ receptor knockout mice, suggesting that a component of ethanol consumption in D₁ receptor knockout mice may be influenced by the A_{2A} receptor. Future studies employing a wider range of paradigms will undoubtedly shed more light on how interactions between D₁ and A_{2A} receptors regulate reward and goal-directed behavior.

D₁ receptor knockout mice demonstrated a reduced consumption of saccharin when compared to wild-type

Table 1 Densitometric Analysis of D₁ Receptor mRNA in Adenosine A_{2A} Receptor Knockout Mice, Compared to Wild-Type Mice

	Wild-type mice (dpm/mm ²)	A _{2A} (-/-) receptor knockout mice (dpm/mm ²)
CPu	1.44 ± 0.04	1.73 ± 0.04*
NAcc	1.19 ± 0.05	1.38 ± 0.04*
OT	1.67 ± 0.06	1.64 ± 0.04

All values represent mean ± SEM.

CPu, caudate-putamen; NAcc, nucleus accumbens; OT, olfactory tubercle.

Significant differences (* indicates $p < 0.05$); t-test or Mann–Whitney rank sum test. $N = 4$ –6 mice per genotype (12 sections per mouse).

mice, but retained a high preference for saccharin over water. This finding is perhaps more consistent with an independent role for dopamine in goal-directed behaviors rather than the processes relaying reward ‘value’, as previously proposed (Cannon and Palmiter, 2003). D₁ receptor mutant mice preferred the saccharin solution, and thus it appears that the absence of the D₁ receptor has not reduced the reward processing for this stimulus, yet a generalized deficit in goal-directed behavior resulted in reduced saccharin-seeking and water consumption, equally. The lack of correspondence between the observed alterations in ethanol and saccharin consummatory behaviors may indicate a separation in the neuronal processes mediating the intake and reinforcement of these two substances. Conversely, the finding that saccharin preference was not altered in D₁ receptor knockout mice may be explained by the nature of the reinforcer—an artificial sweetener. D₁ receptor knockout mice have been demonstrated to possess an intact hedonic response to sucrose (El-Ghundi *et al*, 2003); and as mice respond to saccharin in a similar way to sucrose, saccharin may be considered a sweet and palatable food. The neuronal pathways underpinning food reinforcement may have an increased degree of plasticity and/or redundancy, to ensure species survival. Mice lacking the D₁ receptor from development have needed to respond to food and food-like substances—and perhaps the preserved saccharin preference in D₁ receptor knockout mice is simply because of prior experience with reinforcers of this nature. Support however, for theories suggesting an ‘activational’ role for dopamine (Salamone *et al*, 2003) is provided by these mice, as the lower level of saccharin intake in D₁ receptor-deficient mice was not linked to an alteration in total daily fluid intake (compared to other drinking trials in D₁ receptor knockout mice), but represents a phenotype consistent with an unwillingness to work for the reinforcer (to the extent wild-type mice exhibited).

In contrast to D₁ receptor knockouts, mice lacking both D₁ and A_{2A} receptors exhibited a reduced preference, as well as reduced intake of the saccharin solution compared to wild-type mice. Thus, D₁A_{2A}-deficient mice had reduced consumption and preference of both drug-related (ethanol) and food-related (saccharin) reinforcers. The proposed mechanism by which the A_{2A} receptor is able to influence goal-directed processing is complex, involving an interaction between adenosine and dopamine at the network

level, and may provide the first evidence for the existence of receptor ‘crosstalk’, or neuronal communication at a systems level. In this model, the A_{2A} receptor becomes crucial in modulating D₁ receptor-mediated effects indirectly, via an interaction with D₂ receptors colocalized upon the same neuronal population expressing the A_{2A} receptor. The impairments in goal-directed behavior seen in D₁ receptor-deficient mice were perhaps lessened as compensatory changes in the A_{2A} receptor were able to alleviate the imbalance between D₁ and D₂ receptor processes. Evidence for compensatory mechanisms involving A_{2A} receptors in D₁ receptor-deficient mice includes the upregulation in A_{2A} receptor transcript in the CPu (over 30%), and A_{2A} receptor density increases in the CPu (by over 47%), NAcc, and OT. The level of dopamine in the striatum is crucial for motor function to remain intact (Schultz, 2002). This tonic dopamine availability presumably activates D₂ receptors, and it is by this mechanism that adenosine acting upon A_{2A} receptors may modulate activity—via D₂–A_{2A} heteromeric complexes. Loss of the A_{2A} receptor-mediated fine-tuning of striatal function may result in the further reduction in goal-directed behavior observed in D₁A_{2A} receptor knockout mice. Moreover, a lower saccharin preference and ingestion rate was observed in the D₁A_{2A} receptor knockout, compared to mice deficient in D₁ receptors, with a pattern similar to ethanol intake (wild-type = A_{2A} > D₁ > D₁A_{2A} receptor knockout mice). This again indicates that there may be a contribution from the A_{2A} receptor in mediating goal-directed behaviors.

The phenotype of D₁A_{2A} receptor knockout mice (and the contrast with both wild-type and derivative mutants), whereby preference and consumption rates of ethanol and saccharin were reduced not in a manner predictable from the individual phenotypes of D₁ and A_{2A} receptor-deficient mice, implies an essential role for the interaction between dopamine and adenosine receptors in the regulation of both preference and drive to consume rewarding solutions. Given that D₁ and A_{2A} receptors are largely localized on separate groups of striatal efferents, the clear reward-impaired phenotype of the D₁A_{2A} knockout mice is suggestive of receptor ‘crosstalk’ at the network level that is pertinent to motivational behavior and reinforcement.

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REFERENCES

- Berrendero F, Castane A, Ledent C, Parmentier M, Maldonado R, Valverde O (2003). Increase of morphine withdrawal in mice lacking A_{2A} receptors and no changes in CB₁/A_{2A} double knockout mice. *Eur J Neurosci* 17: 315–324.
- Cannon CM, Palmiter RD (2003). Reward without dopamine. *J Neurosci* 23: 10827–10831.
- Chen JF, Moratalla R, Impagnatiello F, Grandy DK, Cuellar B, Rubinstein M *et al* (2001). The role of the D₂ dopamine receptor (D₂R) in A_{2A} adenosine receptor (A_{2A}R)-mediated behavioral

- and cellular responses as revealed by A_{2A} and D₂ receptor knockout mice. *Proc Natl Acad Sci USA* **98**: 1970–1975.
- Chen F, Rezvani A, Jarrott B, Lawrence AJ (1998). Distribution of GABA_A receptors in the limbic system of alcohol-preferring and non-preferring rats: *in situ* hybridisation histochemistry and receptor autoradiography. *Neurochem Int* **32**: 143–151.
- Djouma E, Lawrence AJ (2002). The effect of chronic ethanol consumption and withdrawal on μ -opioid and dopamine D₁ and D₂ receptor density in Fawn-Hooded rat brain. *J Pharmacol Exp Ther* **302**: 551–559.
- Donnan GA, Kaczmarczyk SJ, McKenzie JS, Kalnins RM, Chilco PJ, Mendelsohn FA (1989). Catecholamine uptake sites in mouse brain: distribution determined by quantitative [³H]mazindol autoradiography. *Brain Res* **504**: 64–71.
- Drago J, Gerfen CR, Lachowicz JE, Steiner H, Hollon TR, Love PE et al (1994). Altered striatal function in a mutant mouse lacking D_{1A} dopamine receptors. *Proc Natl Acad Sci USA* **91**: 12564–12568.
- El-Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T et al (1998). Disruption of dopamine D₁ receptor gene expression attenuates alcohol-seeking behavior. *Eur J Pharmacol* **353**: 149–158.
- El-Ghundi M, O'Dowd BF, Erclik M, George SR (2003). Attenuation of sucrose reinforcement in dopamine D₁ receptor deficient mice. *Eur J Neurosci* **17**: 851–862.
- Ferré S, Fredholm BB, Morelli M, Popoli P, Fuxe K (1997). Adenosine-dopamine receptor-receptor interactions as an integrative mechanism in the basal ganglia. *Trends Neurosci* **20**: 482–487.
- Franklin KBJ, Paxinos G (1997). *The Mouse Brain in Stereotaxic Coordinates*. Academic Press, Inc.: San Diego.
- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr FJ et al (1990). D₁ and D₂ dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* **250**: 1429–1432.
- Irwin S (1968). Comprehensive observational assessment: a systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. *Psychopharmacology* **13**: 222–257.
- Johansson B, Fredholm BB (1995). Further characterization of the binding of the adenosine receptor agonist [³H]CGS 21680 to rat brain using autoradiography. *Neuropharmacology* **34**: 393–403.
- Kawaguchi Y, Wilson CJ, Emson PC (1990). Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. *J Neurosci* **10**: 3421–3438.
- Koob GF (1999). The role of the striatopallidal and extended amygdala systems in drug addiction. *Ann NY Acad Sci* **877**: 445–460.
- Lawrence AJ, Krstew E, Jarrott B (1995). Functional dopamine D₂ receptors on rat vagal afferent neurones. *Br J Pharmacol* **114**: 1329–1334.
- Le Moine C, Bloch B (1995). D₁ and D₂ dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D₁ and D₂ mRNAs in distinct neuronal populations of the dorsal and ventral striatum. *J Comp Neurol* **355**: 418–426.
- Le Moine C, Normand E, Bloch B (1991). Phenotypical characterization of the rat striatal neurons expressing the D₁ dopamine receptor gene. *Proc Natl Acad Sci USA* **88**: 4205–4209.
- Le Moine C, Normand E, Guitteny AF, Fouque B, Teoule R, Bloch B (1990). Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proc Natl Acad Sci USA* **87**: 230–234.
- Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, Vanderhaeghen JJ et al (1997). Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A_{2A} receptor. *Nature* **388**: 674–678.
- McLean KJ, Jarrott B, Lawrence AJ (1996). Neuropeptide Y gene expression and receptor autoradiography in hypertensive and normotensive rat brain. *Mol Brain Res* **35**: 249–259.
- Reiner A, Anderson KD (1990). The patterns of neurotransmitter and neuropeptide co-occurrence among striatal projection neurons: conclusions based on recent findings. *Brain Res Rev* **15**: 251–265.
- Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK et al (2000). Phenotypic characterization of an $\alpha 4$ neuronal nicotinic acetylcholine receptor subunit knock-out mouse. *J Neurosci* **20**: 6431–6441.
- Salamone JD, Correa M, Mingote S, Weber SM (2003). Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry, and drug abuse. *J Pharmacol Exp Ther* **305**: 1–8.
- Schultz W (2002). Getting formal with dopamine and reward. *Neuron* **36**: 241–263.
- Schultz W, Dayan P, Montague PR (1997). A neural substrate of prediction and reward. *Science* **275**: 1593–1599.
- Self DW, Nestler EJ (1995). Molecular mechanisms of drug reinforcement and addiction. *Annu Rev Neurosci* **18**: 463–495.
- Snell BJ, Short JL, Drago J, Ledent C, Lawrence AJ (2000). Characterisation of central adenosine A₁ receptors and adenosine transporters in mice lacking the adenosine A_{2A} receptor. *Brain Res* **877**: 160–169.
- Steiner H, Gerfen CR (1993). Cocaine-induced c-fos messenger RNA is inversely related to dynorphin expression in striatum. *J Neurosci* **13**: 5066–5081.
- Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999). Distribution, biochemistry and function of striatal adenosine A_{2A} receptors. *Prog Neurobiol* **59**: 355–396.
- Svenningsson P, Le Moine C, Kull B, Sunahara R, Bloch B, Fredholm BB (1997). Cellular expression of adenosine A_{2A} receptor messenger RNA in the rat central nervous system with special reference to dopamine innervated areas. *Neuroscience* **80**: 1171–1185.
- Wise RA (1996). Addictive drugs and brain stimulation reward. *Annu Rev Neurosci* **19**: 319–340.