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Emotional response in dopamine D2L receptor-deficient mice

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Abstract

The dopamine D2 receptor (D2R) system has been implicated in emotional processing which is often impaired in neuropsychiatric disorders. The long (D2L) and the short (D2S) isoforms of D2R are generated by alternative splicing of the same gene. To study differential roles of the two D2R isoforms, D2L-deficient mice (D2L^{-/-}) expressing functional D2S were previously generated. In this study the contribution of D2L isoform to emotional response was investigated by examining behaviors that reflect emotionality (exploratory behavior, anxiety-like behavior and learned helplessness) in D2L^{-/-} and (wild-type) WT mice. While the thigmotactic, locomotor and general components of anxiety in zero maze did not differ among the genotypes, D2L^{-/-} mice displayed significantly lower level of exploration in a hole board and zero maze, and significantly higher increase in latency to escape from a foot shock after the learned helplessness training, compared with WT mice. These results suggest that D2L may play a more prominent role than D2S in mediating emotional response, such as behavioral reactions to novelty and inescapable stress. Our findings contribute to a better understanding of the molecular and cellular mechanisms underlying emotional responses.

Keywords

dopamine; D2L knockout mice; emotionality; anxiety; hole board; learned helplessness

Introduction

The dopamine D2 receptor (D2R) system has been implicated in the modulation of motor activity, learning and memory [12,19], as well as in the pathophysiology of neuropsychiatric disorders such as Parkinson's disease (PD) and schizophrenia [14,32].

Several lines of evidence suggest that the D2R system is involved in another important aspect of behavior – emotional processing. Studies using animal models have shown that the activation of D2R in various brain areas is involved in detection of novelty [17], emotional arousal [25], consolidation and retrieval of fear memories [21], and limbic aspects of behavioral responses contributing to the drive of action [3,22].

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Emotional impairments have also been observed in dopamine-related neuropsychiatric disorders. Depression and the lack of behavioral reactivity are often found as comorbidities in PD patients [24,41], and a deficit in dopaminergic transmission in the mesolimbic system is thought to be a part of the cellular mechanisms underlying both impairments [18,22,26]. Psychotic symptoms in patients with schizophrenia are considered to be the result of abnormal emotional processing [11]. The fact that these symptoms are effectively treated with D2R antagonists suggests that the abnormal emotional processing and psychotic ideation observed in schizophrenia are, at least in part, due to the changes in the D2R system. On the other hand, prolonged suppression of D2R activity during treatment with typical antipsychotics can lead to neuroleptic dysphoria [13], pointing to the involvement of D2R in the development of mood disorders frequently observed in schizophrenic patients.

There are two isoforms of D2R: the long form (D2L) and the short form (D2S), which are generated by alternative splicing of the same gene [5,10,20]. The D2L receptor protein has a 29 amino acid insertion in the third cytoplasmic loop – the region considered to be critical for the interaction of the receptor with intracellular effectors [23]. The two D2R isoforms display differential affinities for inhibitory G-proteins [5] and might have differential synaptic locations (D2L mainly postsynaptic and D2S presynaptic, although this issue remains unsettled) [16,35,37], suggesting they have differential physiological functions. Due to the lack of isoform-selective pharmacological agents, studies of differential roles of D2L and D2S in the brain currently rely mainly on genetic manipulation.

To contribute to understanding of functional roles of the two D2R isoforms in the mammalian brain, we have generated D2L receptor-deficient mice (D2L^{-/-}) which only express functional D2S receptors at the level similar to that of the total D2R in wild-type (WT) mice [37]. In the previous studies we showed that D2L^{-/-} mice, in comparison to WT mice, displayed reduced level of locomotion in an open field, lower basal motor activity in their home cages, increased spontaneous immobility, and enhanced stereotyped behavior [7,37]. D2L^{-/-} mice failed to acquire avoidance behavior in response to electrical stimuli [33] and exhibited motor, aggression and learning deficits normally observed in aged mice [8,36]. Moreover, mice lacking D2L were less sensitive to typical antipsychotics [37,39] and failed to develop either a place preference to morphine or a place aversion to morphine withdrawal [33]. On the other hand, the action of the atypical antipsychotic clozapine, the effects of dopamine (DA) on prolactin secretion and dopaminergic neuron firing, the cocaine-induced place preference, and the DA agonist-induced disruption of prepulse-inhibition did not differ between D2L^{-/-} and WT mice [33,37,39,40].

In the present study we focused on the contribution of D2L isoform to emotional response. We compared the behaviors of WT mice expressing predominantly D2L isoform with those of D2L-deficient mice expressing only D2S isoform, using three paradigms that reflect emotionality: exploratory behavior in a hole board, anxiety-like behavior in a zero maze, and behavioral despair after exposure to inescapable shocks in a shuttle box.

Materials and Methods

Animals

Dopamine D2L receptor knockout mice (D2L^{-/-}) were generated by targeted mutagenesis as described previously [37]. Heterozygous mice (D2L^{+/-}) created on a hybrid background (129/Sv × C57BL/6) were backcrossed to the C57BL/6 strain for six generations to establish an incipient congenic B6 line. Male mice (3 months old), obtained by intercross of heterozygous females and heterozygous males, were used in the experiments. The mice were maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room and had access to food and water *ad libitum*. Independent mice were used in different experiments. Experiments were

conducted in an isolated room under ambient light of 30 lux (except for 300 lux in the zero maze test) between 10:00 and 13:00 h by researchers blind with respect to the genotype of the mice. Mice were brought to the experimental room at least 1 h before the onset of experiments to allow adaptation to the environment. Each experimental apparatus was cleaned with 70% ethanol between animals to remove odor cues. Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Hole board

Eight WT and eight D2L^{-/-} mice were tested on a hole board under moderate illumination. The enclosure (40 × 40 × 45 cm) was made of clear Plexiglas walls and a white plastic floor containing 16 holes (3 cm in diameter and 4 cm deep) displayed in a 4 × 4 configuration. Mice were placed in the middle of the hole board and allowed to freely explore for 10 minutes. The enclosure was equipped with photobeam tracking system (San Diego Instruments, San Diego, CA) which recorded interruption of beams under each hole as number of nose pokes.

Zero maze

Eight mice of each genotype (WT, D2L^{+/-}, and D2L^{-/-}) were tested on an elevated zero maze under a bright light for 5 minutes, according to the method described by Shepherd et al. [31]. The zero maze consisted of a 5.5 cm-wide circular track made of white Plexiglas, with inside diameter of 34 cm and elevation of 40 cm. It contained two open quadrants with a raised 2-mm edge and two closed quadrants with 11-cm high walls. Mice were placed in one of the closed quadrants and allowed to investigate the zero maze for 5 minutes. During this time an observer scored mice on several anxiety related variables. The animals were also tracked by a Poly-Track Video System (San Diego Instruments, San Diego, CA), suspended 130 cm above the zero maze. Parameters used in inter-genotype comparisons were percentage of time spent in the open (time that mice spent in open quadrants with all four paws, divided by total testing time), exploratory behavior (number of head dips and rearings), number of transitions (crossings between open and closed quadrants), and total distance (in meters) traveled by mouse over a 5-min period. Grooming and defecation were also scored.

Learned helplessness

Ten mice from each genotype (WT, D2L^{+/-}, D2L^{-/-}) were tested for the learned helplessness (LH), according to a published method [30]. LH training and shuttle escape testing were performed using a Gemini Avoidance System (San Diego Instruments, San Diego, CA), which consisted of control computer and test enclosure. The enclosure (50 × 21 × 17 cm) was made of dark Plexiglas and divided into two equal compartments by a thin aluminum wall. The bottom of the wall contained a mobile gate (9 × 8 cm) which, when lifted, allowed mice to escape from one compartment to the other. The floor was made of stainless steel bars, 2 mm in diameter, spaced 5 mm apart. Position of mice was tracked by the photobeam sensors and shocks were delivered to a grid floor of the occupied compartment. During the LH training, mice were placed in one compartment of the enclosure with the gate closed, and left to habituate for 60 s. Learned helplessness was induced by administering inescapable foot-shock, which consisted of total of 300 shocks (0.30 mA intensity, 2 s duration) delivered at intervals of 10 s. During the shuttle escape testing, mice were placed in the enclosure with the gate open, and allowed to explore the chamber for 5 minutes. Mice were then given 30 shuttle escape trials with 30 s intervals in between. Each trial consisted of a 0.30 mA shock, which was terminated after the mouse escaped into the adjacent compartment, and the time from shock onset to animal escape was recorded as latency to escape (in seconds). If an escape response was not made, the trial was terminated 24 s after a shock onset. For each mouse, shuttle escape testing was performed one week prior to the LH training (initial latency) and 24 hours after the training

session (latency after training). Mice of each genotype were compared for the latency to escape before and after the LH training.

Statistical analyses

Data was processed by the use of GraphPad InStat 3.01 software. Normality of distributions of the measured parameters was tested by Kolmogorov-Smirnov method, while the equality of SDs was tested by Bartlett's test. Mean values of normally distributed parameters were compared using unpaired t-test, or using one-way analysis of variance (ANOVA) or repeated measures ANOVA, both with Tukey's post-hoc test. Mean values of parameters that were not normally distributed were compared using Mann-Whitney U-statistics, or using non-parametric Kruskal-Wallis method or Friedman test, both with Dunn's post-hoc test. The level of significance was set to 0.05. Values were expressed as means $\pm$ standard error of mean (SEM).

Results

Exploratory behavior (hole board)

To assess exploratory behavior WT and D2L^{-/-} mice were examined in a hole board, under a moderate level of illumination. As illustrated in Figure 1, D2L^{-/-} mice displayed significantly lower number of nose pokes than WT mice (39.9 ± 4.4 for D2L^{-/-} and 56.4 ± 3.6 for WT, $t=2.93$, 14 d.f., $p=0.012$), indicating a reduced exploratory behavior in D2L-deficient mice.

Anxiety-like behavior (zero maze)

The level of anxiety in WT, D2L^{+/-} and D2L^{-/-} mice was evaluated in a zero maze using several measures (Fig 2). Locomotor activity, reflected in the number of transitions between open and closed compartments (11.3 ± 2.7 for WT, 6.8 ± 1.3 for D2L^{+/-}, and 6.0 ± 1.6 for D2L^{-/-} mice), and in the total distance traveled (12.1 ± 0.7 m for WT, 10.1 ± 0.6 m for D2L^{+/-}, and 11.7 ± 1.3 m for D2L^{-/-}), did not differ significantly among the genotypes ($F_{(2,23)}=2.11$, $p=0.15$, and $F_{(2,23)}=1.34$, $p=0.28$, respectively). Thigmotactic behavior, measured as percentage of time spent in open quadrants, also did not differ significantly among the genotypes, being 18.4 ± 6.6 % for WT, 13.2 ± 2.8 % for D2L^{+/-}, and 15.5 ± 5.0 % for D2L^{-/-} mice (KW=1.32, $p=0.511$). Similar levels of anxiety were also illustrated by the lack of significant differences in the mean numbers of grooming and fecal boli among the genotypes (KW=0.92, $p=0.63$, and KW=1.87, $p=0.39$, respectively). However, exploratory behavior, measured as the number of head dips and rearings, differed significantly among the genotypes ($F_{(2,23)}=5.76$, $p=0.01$). D2L^{-/-} mice displayed significantly less exploratory behavior (8.0 ± 0.9) than WT mice (14.1 ± 1.7), with the values of D2L^{+/-} mice falling in between (11.4 ± 1.1).

Learned helplessness

Mice of all three genotypes were assessed for the learned helplessness by comparing their latencies to escape from a foot-shock before (initial latency) and after exposure to inescapable shocks (latency after training) using the shuttle box escape test (Fig 3, Fig 4). The initial latencies to escape did not differ among WT (0.98 ± 0.16 s), D2L^{+/-} (1.05 ± 0.10 s) and D2L^{-/-} (1.02 ± 0.16 s) mice, indicating that mice of different genotypes had similar ability to perceive a shock as well as to escape. Although none of the animals displayed a failure to escape after the LH training, an increase in latencies to escape was observed in all three genotypes (Fig 3). An increase in latency of 43% in WT mice was only indicative ($U'=74$, $p=0.09$), whereas increases in latencies of 120% in D2L^{+/-} and of 145% in D2L^{-/-} mice were significant ($t=3.14$, $p=0.01$ for D2L^{+/-}; $U'=93$, $p=0.0005$ for D2L^{-/-}). When the results from

the shuttle escape test were grouped in blocks of 10 trials, the differences in the reaction to inescapable shocks among the genotypes were clearly evident (Fig 4). In WT mice, the latencies to escape only approached significance in the first block of trials ($t=2.11$, $p=0.06$) and then returned to the initial values (i.e. to the latencies prior to the LH training) for the remainder of the experiment. The latencies to escape in D2L^{+/-} and D2L^{-/-} mice tended to decrease over the course of the experiment, but remained significantly increased, compared with the initial values, in all three blocks of trials. These results suggest that D2L-deficient mice seem to be more prone to learned helplessness than WT mice.

Discussion

Exploration and anxiety

Exploration is an important facet of novelty seeking [4] and the nose-poking activity in a hole board is considered a valid index of exploration in rodents [9]. In the present study, D2L^{-/-} mice displayed reduced level of exploratory behavior in a hole board in comparison with WT mice, which could be the result of a reduced response to novelty in mice lacking D2L. However, the reduced level of exploration observed in the mutant mice could have also resulted from increased anxiety or reduced locomotion. The latter possibilities were further explored using the zero maze test. This test allows evaluation of several aspects of anxiety-related behaviors: a) the time spent in open quadrants reflects thigmotactic behavior, b) the number of transitions between the quadrants and total distance traveled reflect locomotor activity of animals in the zero maze environment, and c) the number of head dips and rearings reflect exploratory activity. Thigmotactic behavior and locomotor activity, as well as general anxiety measures (defecation and self-grooming) did not significantly differ among the three genotypes of mice. However, the level of exploration was, again, significantly lower in D2L^{-/-} than in WT mice, with intermediate values in heterozygous mice suggesting possible gene dose-effect.

The neurobiological basis of novelty seeking is not fully understood, but there is evidence indicating the involvement of the dopaminergic system. For example, an increase in dopamine release in the nucleus accumbens and prefrontal cortex has been detected when rats were exposed to a novel environment [6,28]. It is known that the rat strain being sensitive to the DA receptor agonist apomorphine (apo-susceptible) has higher response to novelty than the rat strain being less sensitive to apomorphine (apo-unsusceptible). Upregulation of the dopaminergic system, including an increase in D2R binding sites, have been observed in nigrostriatal and tuberoinfundibular brain regions in the apo-susceptible subline in comparison to the apo-unsusceptible subline [27]. The reduced exploratory behavior in D2L^{-/-} mice observed in our study suggests that the D2L isoform is involved in the exploratory behavior of animals in a novel environment and may modulate the process of detection of or response to novelty.

Learned helplessness

Exposure of animals to aversive stimuli that they cannot control or predict (e.g., inescapable foot-shocks) could impair the animal's ability to react to and escape from the harmful situation, which is termed learned helplessness [29]. The LH paradigm permits assessment of the neurobiological basis of behavioral despair in rodents. Several lines of evidence have implicated the importance of the mesolimbic/nigrostriatal dopaminergic systems in mediating behavioral responses to inescapable stress. It has been demonstrated that D2R density is significantly decreased in the core of the nucleus accumbens and in the medial and lateral caudate nuclei in rats that became "helpless" after the LH training [15]. D2R agonists have been shown to significantly decrease the number of escape failures after the LH training, whereas D2R antagonists have the opposite effect [1,34]. Also, tricyclic antidepressants that increase dopaminergic activity reverse the escape deficit produced by the LH training in rats,

and D2R antagonists were shown to suppress the behavioral effect of tricyclic antidepressants in the LH paradigm [2,34].

In this study, two rounds of shuttle box escape test were performed on WT, D2L^{+/-} and D2L^{-/-} mice. Prior to the LH training, all three genotypes of mice (WT, D2L^{+/-} and D2L^{-/-} mice) had similar escape latencies, indicating that D2L-deficient mice had similar ability to sense a foot shock and to escape from it, to that of WT mice. Also, all genotypes of mice displayed similar reaction to the shock, ranging from vocalization and jumping (at the beginning of the session) to flinching (towards the end of the session), indicating that the mutant mice had a sensitivity to the foot-shock-induced pain similar to that of WT mice. After the LH training, a significant increase in the escape latency throughout the testing session was observed in D2L^{+/-} mice. The effect was even more pronounced in D2L^{-/-} mice, but absent in WT mice in which the escape latencies returned to the initial values in the first half of the session. This suggests that the loss of D2L functionality might have led to the motivational or cognitive deficits that made D2L-deficient animals more prone to learned helplessness.

D2L deficiency vs. D2S overexpression

Previously, we and others have demonstrated using various approaches that D2L^{-/-} mice still express functional D2S isoform on the cell surface at a level similar to that of total D2R in WT mice, due to a compensatory increase in D2S expression in the mutants [7,35,37–40]. The fact that D2L^{-/-} mice have an overall D2 density similar to that of WT mice is experimentally useful; otherwise it would be difficult to discern whether a phenotype was caused by a reduction in total D2 density or by the absence of D2L. On the other hand, a question has been imposed: are behavioral anomalies observed in D2L^{-/-} mice due to the lack of D2L or to the overexpression of D2S? Although we cannot completely exclude the possibility that the increased expression of D2S might have an influence on behavioral phenotypes, we suggest that the behavioral phenotypes shown here were likely due to the lack of D2L for the following reasons. First, if the behavioral phenotypes observed in the mutant are the consequence of increased expression of D2S, then D2S would have a functional effect opposite to that of D2L. As described previously, blockade of D2R using pharmacological agents increases the escape deficits in the LH paradigm in normal rodents [1,2,34]. The fact that mice lacking D2L displayed the enhanced escape deficits in LH paradigm suggested that the deficiencies were likely attributed to the lack of D2L; otherwise the reverse behavioral phenotype would be predicted. Second, studies in transfected cell lines have shown that the two isoforms of D2R either both inhibit adenylyl cyclase, albeit to different degree, or are both coupled to different inhibitory G proteins [5]. This suggests that these two highly structurally homologous proteins function in a complementary and synergistic manner, rather than an antagonistic one. Finally, our previous studies using various experimental paradigms have not indicated that D2L and D2S have functionally opposite actions, although these two receptor isoforms have distinct functions.

We recognize that D2L^{-/-} mouse cannot be used to answer all the questions regarding the roles of D2L. Nevertheless, this mutant mouse provides a useful and unique animal model, which allows us to begin to dissect the specific functions of D2L in the brain in the absence of selective pharmacological agents. Studies using this model, together with other research approaches, will facilitate a better understanding of the functions of individual D2R isoforms - the receptor system that plays an important role in motor control and cognitive functions including emotional processing.

Conclusion

In the present study we investigated the role of D2L receptor isoform in emotional response. We showed that the loss of D2L functionality resulted in a decreased level of exploratory

behavior and higher propensity to behavioral despair, while it had no effect on the level of anxiety-like behavior. These results suggest that D2L may play a more prominent role than D2S in mediating emotional response to environmental stimuli, such as behavioral reactions to novelty and inescapable stress, and support the notion of dopamine as an important integrator of emotion, motivation and action. D2L might be the isoform possibly involved in emotional impairments often seen in dopamine-related disorders, either as comorbidity or as a side effect of pharmacological treatment. Our findings contribute to a better understanding of the neurobiological basis underlying emotional response and its impairments.

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Figure 1.

Exploratory behavior of wild-type (WT) and D2L knockout (D2L^{-/-}) mice, measured as the number of nose pokes in a hole board within 10 minutes of exposure. N=8 per group. Values are expressed as Mean $\pm$ SEM. * $p < 0.05$ (Student's t test).

Figure 2.

Behavior of WT, D2L+/-, and D2L-/- mice in a zero maze. **A)** Numbers of transitions between the quadrants (Transitions), **B)** total distance traveled expressed in meters (Distance), **C)** percentage of time spent in open quadrants (% Open), **D)** exploratory behavior expressed as numbers of head dips and rearings (Exploration), **E)** numbers of grooming, and **F)** numbers of fecal boli were compared among genotypes. N=8 per group. Values are expressed as Mean $\pm$ SEM. ** $p < 0.01$ (Tukey-Kramer post-ANOVA test).

Figure 3. Mean latencies to escape from a foot-shock (in seconds), obtained from 30 trials, before and after exposure to inescapable shocks in WT, D2L+/-, and D2L-/- mice. N=10 per genotype. Values are expressed as Mean $\pm$ SEM. ** $p=0.01$ (Student's t test), *** $p<0.001$ (Mann-Whitney test).

Figure 4.

Latencies to escape from a foot-shock (in seconds), plotted as three blocks of ten trials, before (open diamonds) and after (closed squares) exposure to inescapable shocks in: **A)** WT mice, **B)** D2L+/- mice, and **C)** D2L-/- mice. N=10 per genotype. Values are expressed as Mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Mann-Whitney test).