Progress in Neuro-Psychopharmacology & Biological Psychiatry 33 (2009) 330–333

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnpbp

Phenotype of spontaneous orofacial dyskinesia in neuregulin-1 'knockout' mice

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ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form 9 December 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Knockout mice
Neuregulin-1
Orofacial dyskinesia
Phenotype
Schizophrenia
Targeted gene deletion

ABSTRACT

Studies in antipsychotic-naïve patients with schizophrenia indicate a baseline level of spontaneous involuntary movements, particularly orofacial dyskinesia. Neuregulin-1 is associated with risk for schizophrenia and its functional role can be studied in 'knockout' mice. We have shown previously that neuregulin-1 'knockouts' evidence disruption in social behaviour. Neuregulin-1 'knockouts' were assessed for four topographies of orofacial movement, both spontaneously and under challenge with the D_1-like dopamine receptor agonist SKF 83959. Neuregulin-1 'knockouts' evidenced an increase in spontaneous incisor chattering, particularly among males. SKF 83959 induced incisor chattering, vertical jaw movements and tongue protrusions; the level of horizontal jaw movements was increased and that of tongue protrusions decreased in neuregulin-1 'knockouts'. These findings indicate that the schizophrenia risk gene neuregulin-1 is involved in the regulation of not only social behaviour but also orofacial dyskinesia. Orofacial dyskinesia in neuregulin-1 mutants may indicate some modest genetic relationship between risk for schizophrenia and vulnerability to spontaneous movement disorder.

1. Introduction

While movement disorder in patients treated with antipsychotic drugs is recognised as a side effect of such medication, a critical debate endures: to what extent is movement disorder intrinsic to the disease process of schizophrenia? For example, involuntary movements are widely recognised to occur to excess in schizophrenia but have been interpreted primarily as an adverse effect of long-term treatment with antipsychotic drugs, i.e. tardive dyskinesia, rather than an intrinsic feature of, and hence informative on, the disease process. However, studies in antipsychotic-naïve patients clearly indicate spontaneous movement disorder, both extrapyramidal phenomena such as Parkinsonism (Chatterjee et al., 1995; Cortese et al., 2005; Whitty et al., 2008) and particularly involuntary movements such as orofacial dyskinesia (Waddington, 1989; Bocti et al., 2003; Whitty et al., 2008).

Several genes have now been associated with risk for schizophrenia (Harrison and Weinberger, 2005; Gogos, 2007; Waddington et al., 2007). As the functional role of many of these genes is unclear, targeted deletion ['knockout'] has been applied to generate mutant mice that can inform on their phenotypic roles (Arguello and Gogos, 2006; O'Tuathaigh et al., 2007a; Waddington et al., 2007). Among these genes, neuregulin-1 [NRG1] is associated with risk for schizophrenia (Harrison and Law, 2006; Li et al., 2006; Munafo et al., 2006) and has been deleted in mice (Stefansson et al., 2002; O'Tuathaigh et al., 2006). We have developed a novel technique for assessing individual topographies of orofacial movement in mice (Tomiyama et al., 2001). Here, we have applied this to NRG1 mutants and report spontaneous orofacial dyskinesia and disrupted effects of SKF 83959, a D_1-like dopamine receptor agonist known to induce orofacial dyskinesia (Waddington et al., 2005).

2. Methods

2.1. Subjects

Transmembrane [TM]-domain NRG1 'knockout' mice were generated at the Victor Chang Cardiac Research Institute, University of New South Wales, Australia, as described previously (Stefansson et al., 2002) and maintained on a C57BL6 background [14 backcrosses (O'Tuathaigh et al., 2006, 2008)]. While homozygous NRG1 mutants die prenatally due to cardiac defects, heterozygous NRG1 mutants are viable and fertile. As described previously in detail (O'Tuathaigh et al., 2006, 2008), heterozygous NRG1 mutants [$NRG1^{+/-}$] and wildtypes [WT; $NRG1^{+/+}$] were generated from heterozygous breeding pairs and offspring genotyped using PCR. Mice were housed in groups of 3–5 per cage and maintained on a standard 12:12 h light:dark cycle [08:00 on; 20:00 off] with ad libitum access to food and water. These studies were approved by the Animal Experimentation Committee of Nihon

Abbreviations: ANOVA, analysis of variance; NRG1, neuregulin-1; PCR, polymerase chain reaction; TM, transmembrane.

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doi:10.1016/j.pnpbp.2008.12.010

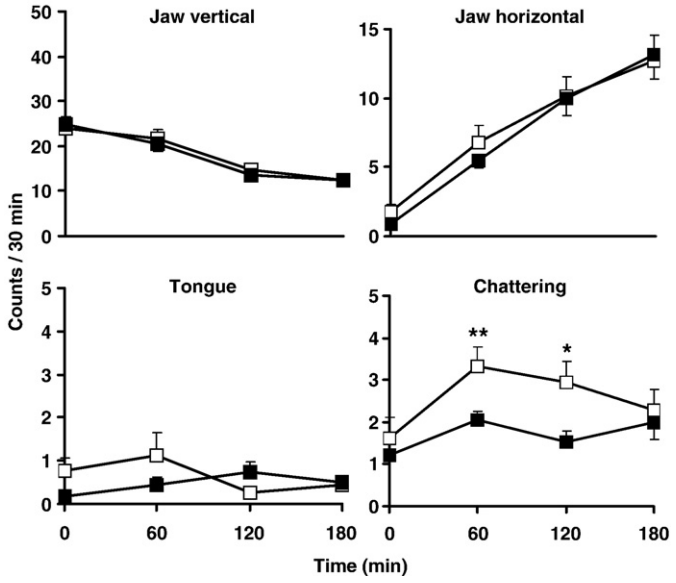

Fig. 1. Topography of orofacial movements in neuregulin-1 mutants (open squares; $n=18$ [11 male, 7 female] per group) and wildtypes (filled squares; $n=22$ [9 male, 13 female] per group). Data are mean counts±SEM for vertical and horizontal jaw movements, tongue protrusions and incisor chattering over 30 min periods beginning at 0, 60, 120 and 180 min after placement in the apparatus. *$P<0.05$, **$P<0.01$ vs wildtypes.

University School of Dentistry, Tokyo, and the Research Ethics Committee of the Royal College of Surgeons in Ireland, Dublin. They were conducted under licence from the Department of Health and Children in accordance with Irish legislation and the European Communities Council Directive 86/609/EEC for the care and use of experimental animals, and from the Environmental Protection Agency in relation to the contained use of genetically modified organisms.

2.2. Assessment

As described previously in detail (Tomiyama et al., 2001, 2004, 2006), mice were placed in a restrictor and a rapid time-sampling behavioural checklist applied to resolve four topographies of orofacial movement: vertical jaw movements; horizontal (lateral) jaw movements; tongue protrusions; and chattering (high-frequency rhythmical jaw movements with incisor tapping).

For spontaneous orofacial movements, male and female mice were observed over 0–30, 60–90, 120–150 and 180–210 min after placement in restrictors. Each of five mice was observed sequentially for 5 s periods at 25 s intervals, with the presence or absence of each individual topography of orofacial movement (occurring alone or in any combination) determined in each of the 5 s periods; thus, the presence of individual topographies was determined in 72 time bins of 5 s over each 30 min period. Mice were used on a single occasion only.

For drug studies, male mice were used in accordance with, and to facilitate reference to, our previous drug studies conducted in males (Tomiyama et al., 2001, 2004, 2006). Mice were habituated to restrictors for 3 h before treatment with drug or vehicle and orofacial movements then determined in 144 time bins of 5 s over a 60 min period. To conserve animals, mice were studied on two occasions only, separated by a drug-free interval of at least one week and with random allocation to treatment on each occasion. In all experiments, the observer was blind to genotype and treatment for each animal.

2.3. Drugs

The drug used was SKF 83959 ([*R*/*S*]-3-methyl-6-chloro-7,8-dihydroxy-1-[3-methyl-phenyl]-2,3,4,5-tetrahydro-1*H*-3-benzazepine; RBI/SRI-NIMH Chemical Synthesis Program, USA), dissolved in distilled water. Injections of drug or vehicle were subcutaneously administered into the flank in a volume of 2 ml/kg.

2.4. Analysis

Total 'counts' for each topography of orofacial movement were the number of 5 s time bins in which a given behaviour was evident, summed over the indicated time periods and expressed as means±SEM. Counts for spontaneous orofacial movements at each time point were compared between NRG1 and WT using the Mann–Whitney *U*-test. Counts for drug-induced orofacial movements were compared across groups using the Kruskal–Wallis non-parametric analysis of variance (ANOVA) and compared between NRG1 and WT at each dose using the Mann–Whitney *U*-test.

3. Results

3.1. General parameters

On examining 18 [11 male, 7 female] NRG1$^{+/-}$ mice, mean age and body weight [185±24 days; 23±1 g] did not differ significantly from 22 [9 male, 13 female] wildtypes [222±22 days; 25±1 g].

3.2. Spontaneous orofacial movements

NRG1 mutants showed an excess of incisor chattering at 60–90 min [$P<0.01$, Mann–Whitney *U*-test] and 120–150 min [$P<0.05$, Mann–Whitney *U*-test] (Fig. 1). This effect was more evident in males than in females; male mutants showed increased chattering relative to wildtypes [$P<0.05$ at 60–90 min, Mann–Whitney *U*-test] while female mutants did not.

Decrease in vertical jaw movements and increase in horizontal jaw movements over time bins were unaltered. Spontaneous tongue protrusions were too few for meaningful analysis.

3.3. Orofacial movements induced by SKF 83959

In male mice, SKF 83959 induced incisor chattering and vertical jaw movements [each $P<0.05$, Kruskal–Wallis ANOVA for both NRG1 and WT] but not horizontal jaw movements; at 0.4 mg/kg, horizontal

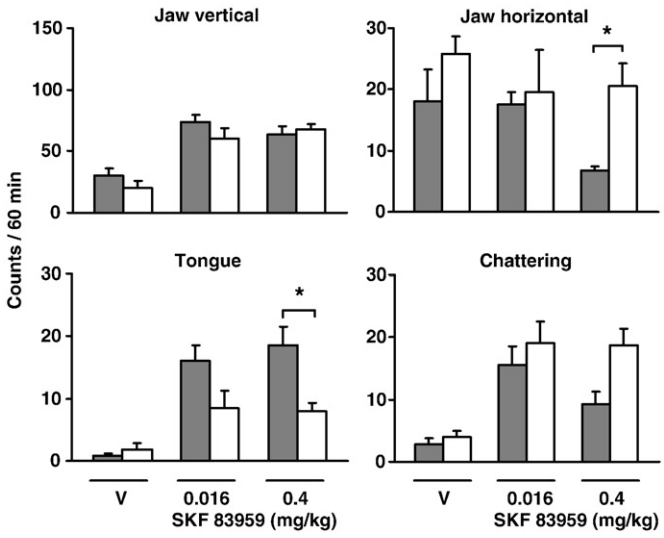

Fig. 2. Topography of orofacial movements in neuregulin-1 mutants (open columns; $n=4$–5 males per group) and wildtypes (filled columns; $n=4$–5 males per group) following challenge with 0.016–0.4 mg/kg SKF 83959 or vehicle. Data are mean counts ±SEM for vertical and horizontal jaw movements, tongue protrusions and incisor chattering over a 60 min period after drug challenge, following habituation to the apparatus. *$P<0.05$ vs wildtypes.