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

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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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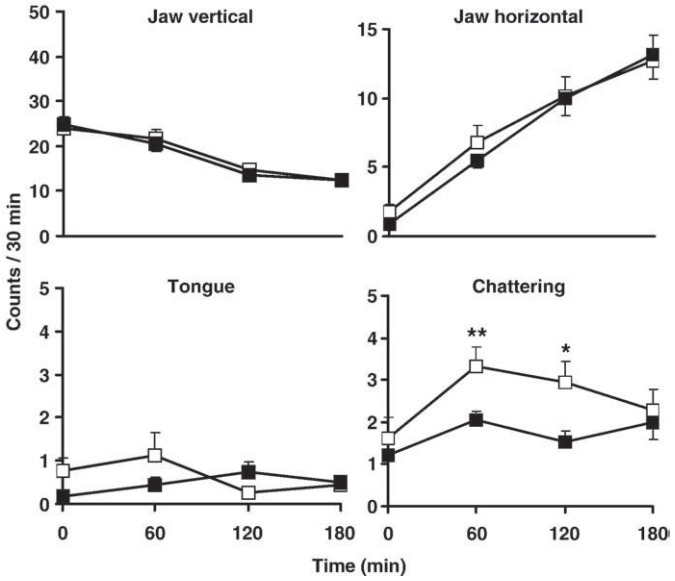

Fig. 1. Topography of orofacial movements in neuregulin-1 mutants (open squares; $n=18$ [11 male, 7 female] per group) and wildtypes (filled squares; $n=22$ [9 male, 13 female] per group). Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue protrusions and incisor chattering 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

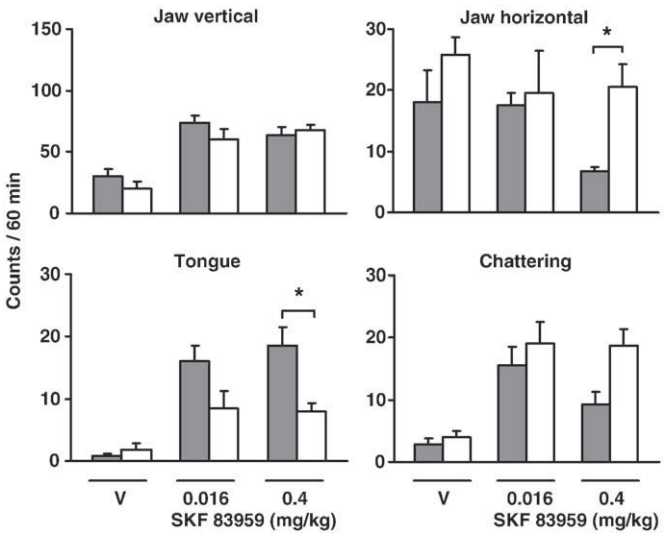

Fig. 2. Topography of orofacial movements in neuregulin-1 mutants (open columns; $n=4$–5 males per group) and wildtypes (filled columns; $n=4$–5 males per group) following challenge with 0.016–0.4 mg/kg SKF 83959 or vehicle. Data are mean counts ± SEM for vertical and horizontal jaw movements, tongue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

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jaw movements were higher in NRG1 mutants than in WT [$P<0.05$, Mann–Whitney U-test] (Fig. 2).

SKF 83959 also induced tongue protrusions [$P<0.05$, Kruskal–Wallis ANOVA for both NRG1 and WT]; at 0.4 mg/kg, tongue protrusions were lower in NRG1 mutants than in WT [$P<0.05$, Mann–Whitney U-test].

4. Discussion

Our main findings can be summarized as follows: (i) heterozygous NRG1 'knockouts' evidence an increase in spontaneous incisor chattering; (ii) during challenge with a high dose of SKF 83959, NRG1 mutants evidence an increased level of horizontal jaw movements; and (iii) during challenge with a high dose of SKF 83959, NRG1 mutants evidence a reduced level of tongue protrusions.

In WT mice, the overall profile of spontaneous orofacial movements [decrease in vertical jaw movements and increase in horizontal jaw movements with interpolated emergence of chattering] is as reported in several previous studies using the present paradigm (Tomiyama et al., 2001, 2004, 2006; Waddington et al., 2005); this profile likely reflects an interaction between the ethology of murine orofacial movements and initial stress associated with a restrictor system necessary to allow resolution and quantification of individual topographies of orofacial movements that cannot be accessed using naturalistic procedures. In WT mice, the overall profile of response to SKF 83959 [induction of vertical but not horizontal jaw movements, together with induction of incisor chattering and tongue protrusions] is also as reported in several previous studies using this paradigm (Tomiyama et al., 2001, 2004, 2006; Waddington et al., 2005). In accordance therewith, spontaneous orofacial movements were assessed over the course of habituation to the restrictor system, so as to define the interplay between the ethology of murine orofacial movements and initial stress associated with a restrictor system; drug challenge studies were then conducted, so as to define the effects of drug vs vehicle on the baseline consequent to habituation. These previous findings and associated methodological issues have been considered in detail elsewhere (Tomiyama et al., 2001, 2004, 2006; Waddington et al., 2005).

Incisor chattering involves rhythmical jaw movements with incisor tapping. Their excess in NRG1 'knockouts' indicates that spontaneous, topographically specific orofacial dyskinesia results from deletion of this gene, which has been associated with risk for schizophrenia (Harrison and Law 2006; Li et al., 2006; Munafo et al., 2006) and regulation of social behaviour (O'Tuathaigh et al., 2007b, 2008). The restrictor system is likely to be stressful, at least initially (Tomiyama et al., 2001) and [tardive] orofacial dyskinesia in schizophrenia can be exacerbated by stress (Kane and Smith, 1982; Waddington, 1989).

Importantly, any specific relationship between individual topographies of orofacial movement in NRG1 mutant mice and those constituting tardive dyskinesia is not clear. Humans have a much more complex repertoire of orofacial movements than do mice, to include verbal communicative and expressive as well as consummatory functions. This may vitiate attempts to make more precise clinical interpretations of murine phenotypic data. Furthermore, as for all conventional 'knockouts' (Waddington et al., 2005), the NRG1 mutant phenotype may be influenced by compensatory mechanisms arising over the course of development.

Previous studies in NRG1 mutants have indicated a 'hyperactive' phenotype (see O'Tuathaigh et al., 2006, 2007a), with no evidence for Parkinsonian or related features; these included sex-specific phenotypic effects, as encountered previously in a number of other 'knockouts' (see Waddington et al., 2005). Thus, the present sex-dependent aspects of orofacial phenotype in NRG1 mutants, relating to increased spontaneous chattering primarily among males, constitute further examples. It has been reported that aspects of tardive dyskinesia in schizophrenia can vary between the sexes (Kane and Smith, 1982; Waddington, 1989).

Given the recognised role of D_1-like receptors in orofacial movements (Waddington et al., 2005), we challenged NRG1 mutants with SKF 83959, a D_1-like agonist that induces such movements (Tomiyama et al., 2001, 2006). At a high dose of SKF 83959, NRG1 mutants evidenced an increased level of horizontal jaw movements and a decreased level of tongue protrusions. We have previously reviewed evidence that horizontal jaw movements and tongue protrusions evidence overlapping but not identical pharmacological profiles and are therefore presumably subserved by overlapping but not identical mechanisms (Waddington et al., 2005). Phenotypic effects at the levels of spontaneous and D_1-like agonist-induced behaviour in NRG1 mutants may be distinct and bear differing relationships to tardive dyskinesia. Future studies should include more detailed pharmacological characterization of the orofacial phenotype of NRG1 mutants and extend this to include the effects of acute and chronic administration of D_2-like antagonists.

5. Conclusions

NRG1 is expressed in several brain regions, including the basal ganglia, and putative functional roles for NRG1 include synapse formation, neuronal migration, synaptic plasticity and the regulation of neurotransmitter expression and release (Harrison and Law, 2006). Additionally, NRG1 is a replicable risk gene for schizophrenia (Harrison and Law, 2006; Li et al., 2006; Munafo et al., 2006; Waddington et al., 2007), though there is no evidence for a haploinsufficiency of NRG1 in patients. Studies in antipsychotic-naïve patients with schizophrenia indicate spontaneous involuntary movements, particularly orofacial dyskinesia, to be at least in part a component of the disease process (Waddington, 1989; Bocti et al., 2003; Whitty et al., 2008). Most of those patients with involuntary movements are unlikely to carry a risk NRG1 haplotype. More extensive studies are necessary to clarify the mechanistic basis of these phenotypic effects, which involve not only disruption to social behaviour but also the presence of orofacial dyskinesia in NRG1 mutants. Thus, the present findings suggest some modest genetic relationship between risk for schizophrenia and vulnerability to spontaneous involuntary movement disorder.

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