Genetic Mapping and Evaluation of Candidate Genes for Spasmodic, a Neurological Mouse Mutation with Abnormal Startle Response

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Spasmodic (spd) is a recessive mouse mutation characterized by a prolonged righting reflex, fine motor tremor, leg clapping, and stiffness. Using an intersubspecific backcross that segregates spd, we placed spd on Chr 11 with the following gene order: Adra-1–3.8 ± 2.1 cm–Pad-1–6.3 ± 2.7–(spd, Anx-6, Csflgm, Glr-1, Il-3, Il-4, Il-5, Sparc)–9.1 ± 2.4–D11Mit5–2.2 ± 1.5–Asgr-1. This localization eliminated the α1-adrenergic receptor (Adra-1) and the α1 and γ2 subunits of the GABA_A receptor as candidate genes. Two other promising candidate genes, annexin VI (Anx-6) and a glutamate receptor (Glr-1), were mapped to within 2.1 cm of the spd locus. Although no recombination was observed between spd and Anx-6 or Glr-1, no evidence was obtained for a lesion in either gene. The presence of normal Anx-6 and Glr-1 mRNA transcripts was confirmed by Northern blot analysis, in situ hybridization, and DNA sequence analysis. The localization of Anx-6 and Glr-1 extends the known synteny homology between human chromosome 5q21–q31 and mouse Chr 11 and reveals the probable chromosomal location of the human counterpart to spd. Synteny homology and phenotypic similarities suggest that spasmodic mice may be a genetic model for the inherited human startle disease, hyperekplexia (STHE). © 1993 Academic Press, Inc.

INTRODUCTION

Spasmodic (spd) is a spontaneous mouse mutation that arose on an A/HeJ background in 1979 at The Jackson Laboratory (Lane et al., 1987). spd segregates as a recessive single gene defect with a complex neurological phenotype. Mice homozygous for this mutation exhibit a fine motor tremor and clasp their hind legs when held by the tail. Several other aspects of their phenotype, including stiffness, a toe-walking spastic gait, and prolonged righting reflex, vary in severity among individual mice and are exacerbated when the mice are disturbed. In fact, undisturbed spd/spd mice can appear normal. Preliminary neuroanatomical studies reveal no obvious abnormalities in brains of spd/spd mice and no alterations in the major myelin proteins and lipids (Lane et al., 1987).

Pharmacological studies suggest that the defect in spd/spd mice could be a deficiency in inhibitory neurotransmission or an excess of excitatory neurotransmission. GABA (γ-aminobutyric acid) and glycine are the major inhibitory neurotransmitters in the brain, and normal mice given subconvulsive doses of the glycine receptor antagonist strychnine display a phenotype that resembles that of spasmodic mice (Heller et al., 1982). Moreover, the phenotype of the spd/spd mutants is ameliorated with aminoxyacetic acid (AOAA), an agent that inhibits the degradation of the inhibitory neurotransmitter GABA. The spd mutation may directly or indirectly affect a component of the glycnergic or gabaergic systems. The interdependence of neurotransmitter systems and the pleiotropic action of many drugs make it difficult to narrow the field of candidate genes with additional pharmacological studies. A genetic approach offers a powerful mechanism for unambiguously assessing candidate genes.

Classical genetic studies placed spd on Chr 11 (Lane et al., 1987). Three neurotransmitter receptor genes warranted further consideration as candidates for spd by their localization on Chr 11 (Buckwalter et al., 1991; 1992; Oakey et al., 1991). Adra-1, the α1-adrenergic receptor gene, is involved in noradrenergic neurotransmission. The leg-clasping and muscle rigidity characteristic of spasmodic mice (Fig. 1) can be produced in normal rodents by alterations in noradrenaline levels (Mogilnicka et al., 1983). Gabra-1 and Gabrg-2, the α1 and γ2 subunit genes of the GABA_A receptor, are excellent candidates because of the amelioration of the spasmodic phenotype by AOAA (Lane et al., 1987). The ambiguity in comparing independent maps necessitated testing these genes for cosegregation with spd.

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS). Glutamate receptors are postulated to play roles in excitation-induced neuronal cell death and neurodegenerative disorders (Michae-
FIG. 1. Muscle rigidity and leg-clasping are characteristic of spasmodic mice. When suspended in the air normal mice arch their back, spread their feet and attempt to right themselves (left). Spasmodic mice become rigid and clasp their hind legs (right).

lis et al., 1992). At least 20 glutamate receptor genes are known to be expressed in the CNS. The human Glr-1 gene, also known as GluR-K1 (Hollmann et al., 1989), GluHI and GluR1 (Puckett et al., 1991), and GluR-A (Keinanén et al., 1990), has been mapped to chromosome 5q33 (Puckett et al., 1991), and syntenic homology suggests that the mouse gene would map on mouse Chr 11 or 18 (Westbrook et al., 1992). Thus, the role of Glr-1 in excitatory neurotransmission and the likelihood of linkage conservation suggested Glr-1 as a candidate gene for spd.

The important role of calcium in neurotransmission suggested the merit of examining candidate genes such as annexin VI (Anx-6). Anx-6 exhibits calcium-dependent binding to membranes, and it is implicated in endocytosis (Lin et al., 1992) and regulation of the ryanodineresitive Ca$^{2+}$ channel (Díaz-Muñoz et al., 1990). Anx-6 is expressed in many cell types, including lymphocytes and neurons, but not glial cells (Owens et al., 1984; Woolgar et al., 1990). As a membrane binding protein probably involved in calcium-dependent endocytosis, Anx-6 may be, either directly or indirectly, involved in release of neurotransmitter vesicles from neurons or neurotransmitter uptake from synapses. Prior to this study, Anx-6 had been assigned to Chr 11 with the use of somatic cell hybrids, under the name of p68 calcium-binding protein (Cabm), but not localized (Davies et al., 1989).

In this paper we report additional characterization of the spd/spd phenotype, localization of spd with respect to 11 molecular markers, assessment of Adra-1, Gabrg-2, Gabra-1, Glr-1, and Anx-6 as candidates for spd, and we discuss evidence that spd may be a mouse model for the human disease hyperkplexia (STHE).

MATERIALS AND METHODS

Mice. Mice carrying the spasmodic mutation (B6C3Fe-a/a-spd/spd) and Mus musculus castaneus mice (inbred strain CASA/Rh) were purchased from The Jackson Laboratory (Bar Harbor, ME). Female B6C3Fe-a/a-spd/spd mice were mated to CASA/Rh males and the male F1 progeny were backcrossed to B6C3Fe-a/a-spd/spd females. Progeny of the backcross were killed at 4–8 weeks of age. Backcross progeny were classified as spd/spd if they displayed any of the following characteristics: a fine motor tremor, clasp of the hind legs when suspended by the tail, or delayed righting reflex. C57BL/6J and C3HeB/Feh mice, purchased from The Jackson Laboratory, were bred to produce F1 mice to serve as normal controls.

All experiments were approved by the University of Michigan Committee on Use and Care of Animals and all animals were housed and cared for according to NIH guidelines.

Electrocortical recordings. Mice were anesthetized with avertin (1.25% tribromoethanol/amyl alcohol solution) by intraperitoneal injection (0.02 ml/g). Silver wire electrodes (0.061-in. diameter) soldered to a microminiature connector were implanted bilaterally into the subdural space over frontal and parietal cortex. Electrocortical activity was recorded 24 h after surgery on mice moving freely in the test cage using a Grass Model 6 electroencephalograph. Ketamine was administered at 100 mg/kg.

Isolation of DNA and RNA. Approximately 3 cm of tail tissue was biopsied from 145 backcross animals, and a salt extraction procedure (Miller et al., 1988) was used to isolate high-molecular-weight genomic DNA. Liver, kidney, lung, and spleen from each animal were snap-frozen in liquid nitrogen and stored at −70ºC. High-molecular-weight DNA was prepared from frozen organs as described (Buckweller et al., 1991). RNA was isolated from whole brains by homogenization in guanidinium thiocyanate and centrifugation through a CaCl2 gradient (Chirgwin et al., 1979). Polyadenylated RNA was affinity purified by oligo(dT)-cellulose chromatography.

Locus mapping. Allele typing at Anx-6, Glr-1, Adra-1, Csf2m/H-3 (granulocyte-macrophage colony stimulating factor and interleukin 3), Il-5 (interleukin 5), Pad-1 (a mouse mammary tumor virus insertion site), Sparc (osteomodulin), and Asgr-1 (asialoglycoprotein recep-
tor-1) was performed with Southern blots (Buckweller et al., 1991). CF4.2, a full-length murine annexin VI cDNA, was donated by Stephen E. Moss, University College, London (Moss et al., 1988). To generate a Glr-1 probe, cDNA was synthesized from B6C3Fe-a/a-spd/spd and (C57BL/6J × C3HeB/FeJ)F1 brain RNAs with reverse transcriptase (avian myeloblastosis virus reverse transcriptase, Seikakagak America, Inc.) and amplified with the primers K1 and K2, 5'-TCA-GCCAGATTCCCAAGCAG-3' and 5'-CTGTTGGCTAGCCCCAACTC-
T-G (Puckett et al., 1991). Amplified product, corresponding to amino acids 135 to 305 of the 889 amino acid protein, was excised from an agarose gel and used directly for labeling. Probes used for typing Adra-1, Csf2m/H-3, Sparc, and Asgr-1 have been described previously (Buckweller et al., 1991). Allele typing at Il-4 (interleukin 4), Glus (glutamine synthetase), D11Mit1, and D11Mit25 was accomplished by PCR (Buckweller et al., 1992; Love et al., 1990).

Statistical analysis. Genetic distances are given in centimorgans with the estimated standard deviation and 95% confidence intervals calculated as described previously (Buckweller et al., 1991). When no recombinant events are observed, the 95% confidence interval of the distance between the two markers is calculated as in Lyon and Seerle (1989).

Cloning and sequencing. Anx-6 cDNA was amplified in two overlapping parts. Nucleotides 17 to 1308 of the cDNA were amplified with the oligonucleotides 5'-CCGGATCCATGCCGGTGTCTTCCTGC-
GC-3' and 5'-GGTCTAGAGAGCCAGAATCTACGTCT GC-3', and nucleotides 1211 to 2355 were amplified with the primer pair 5'-CCGG-
ATCCATGCACATCTGTCACACC-3' and 5'-GGTCTAGAG-
TGCAAAAGCTCCAAGTCCAG-3' (Moss et al., 1988). Amplified products were purified by agarose gel electrophoresis, digested with BamHI and XbaI restriction endonucleases (Bethesda Research Laboratories), and ligated into pUC18 plasmid. Sequencing was performed with M13 forward and reverse primers (Boehringer Mannheim Biochemicals) and 15-hp primers corresponding to nucleotides 224–258, 254–288, 514–528, 739–744, 1083–1097, 1499–1502, 1779–1784, and 2135–2167.

Segments of the Glr-1 cDNA corresponding to nucleotides 206 to
RESULTS

Electrocortical activity in spd homozygotes. Direct electrocortical recordings from chronically implanted spasmodic mice showed that the amplitudes and frequencies of the baseline resting EEG activity in adult homozygotes were unremarkable when compared with those from normal control mice, and no abnormal spontaneous discharges were noted during exploratory behavioral activity (Fig. 2). We observed an unusual reaction during anesthesia in spasmodic mice. Administration of ketamine dramatically enhanced several neurological features of spasmodic mice, including a greatly exagger-
FIG. 3. Haplotype analysis of 88 backcross progeny demonstrate that spd is between Adra-1 and Asgr-1, distal to Pad-1. Analysis of the individuals exhibiting recombination between Adra-1 and spd resulted in the unambiguous gene order Adra-1-Pad-1-spd-Asgr-1. All other gene orders result in multiple double crossover events. The number of animals observed with each haplotype is listed below the schematic of the chromosome. The genotypes spd/spd and spd/+ are depicted at each locus tested (left) by open and closed boxes, respectively.

We tested the first few backcross progeny with several markers on Chr 11 to determine which interval contained the spd locus. After localizing spd to the region between Adra-1 and Asgr-1, we employed an interval mapping strategy that focused on recombinants between these two loci (Fig. 3). Results of this analysis unambiguously placed spd distal to Pad-1.

To increase efficiency, later typings were performed by PCR amplification of simple sequence repeat polymorphisms at a different pair of flanking markers, Glrs and D11Mit5, which map 19.5 ± 6.2 cm proximal and 9.1 ± 2.4 cm distal to spd, respectively. Thirty-one recombinant progeny identified by interval mapping with this pair and the previous pair of flanking markers were typed with additional markers. No recombinants were observed between spd and CsfgrmII-3, II-4, II-5, and Sparc. The gene order and genetic distances observed in this cross (Fig. 4) are consistent with those generated in another intersubspecific backcross with M. m. castaneus (Buckwalter et al., 1991) and with the consensus map of Chr 11 (Buchberg et al., 1992).

Three neurotransmitter genes were considered as candidate genes for spd: Adra-1, Gabrb-1, and Gabrb-2. Adra-1 was mapped 9.3 ± 1.9 cm proximal to spd. The detection of numerous recombination events between Adra-1 and spd demonstrates that they are distinct loci. Adra-1 and Gabrb-1 as a candidate gene because Adra-1 and Gabrb-2 were considered especially good candidates for spd because they are part of the gabaergic neurotransmitter system. We mapped these two genes 4.3 ± 1.9 cm proximal to Pad-1 (Buckwalter et al., 1992), and spd distal to Pad-1 (Fig. 4). The placement of spd in a different interval of the chromosome from that containing Gabrb-1 and Gabrb-2 eliminated them as candidates for spd.

Analysis of Anx-6 as a candidate gene for spd. We used Southern blots to genotype animals at Anx-6 (Fig. 5). No recombination was observed between the spd locus and this gene in 143 backcross progeny, localizing Anx-6 to within 2.1 cm (95% confidence interval) of spd. Northern blots of whole brain RNA from spasmodi and control (C57BL/6J × C3HeB/FeJ)F1 mice demonstrated that Anx-6 mRNA was present in spasmodi mice in normal quantities (Fig. 6). Transcript size appeared to be 2.4 kb, unaltered relative to normal. Therefore, it is unlikely that spasmodi mice carry a defect in Anx-6 that affects gene transcription, mRNA stability, splicing, or polyadenylation.

To screen for point mutations, mRNA from spasmodi and control (C57BL/6J × C3HeB/FeJ)F1 mice

FIG. 4. Comparison of genetic maps of mouse chromosome 11. Haplotype analysis and minimization of crossover frequency were used to determine genetic distances in backcrosses segregating spd (left) and df (right) (Buckwalter et al., 1991). Eleven individual loci were mapped on both crosses (center). Gabrb-1 and Gabrb-2 were mapped on the df cross (Buckwalter et al., 1992). No recombination was observed between Adra-1, Gabrb-1, and Gabrb-2 in 96 animals. Genetic distances and the estimated standard deviation (cm) are reported for each interval. The human chromosomal locations of each locus group are listed (GDB), except for Pad-1, spd, and D11Mit5, which are unknown.

FIG. 5. Southern blot identification of RFLPs in the Anx-6 and Glr-1 genes. Genomic DNA from CASA/Rk mice (C), (CASA/Rk X B6C3Fe-a/a-spd/spd)F1 mice (F), and B6C3Fe-a/a-spd/spd mice (S) was digested with the indicated restriction enzyme (bottom) and hybridized with cDNA probes for Anx-6 (left) or Glr-1 (right). Closed arrowheads indicate the molecular weights, in kb, of hybridizing restriction fragments unique to CASA/Rk DNA, and the open arrowhead indicates a fragment unique to B6C3Fe-a/a-spd/spd DNA.
was reverse transcribed and amplified with Anx-6-specific primers. Clones comprising the entire coding region and all but 17 bp of the 5' and 45 bp of the 3' untranslated regions of Anx-6 were isolated and sequenced from each mRNA sample. Sequences were compared to the published mouse sequence (Moss et al., 1988; Accession No. X13460). Four potential basepair substitutions in the cloned sequences were found to be PCR or cloning artifacts by directly sequencing a pool of PCR products. One silent change was noted in the coding sequence, a G to an A at nucleotide 831. This change probably represents strain variation from the published Anx-6 sequence because it was also present in the sequence we obtained from the F1 control mice. In the 3' untranslated region, five base differences were noted between spd and the published sequence of Anx-6, including G to A changes at nucleotides 2056, 2083, and 2149, an insertion of a G after nucleotide 2213, and an A to C change at nucleotide 2284. These differences are attributable to strain variation because they were found in the F1 control mice. Thus, there is no evidence for mutations in the coding region or 5' and 3' untranslated regions of Anx-6.

**Evaluation of G1r-1 as a candidate gene for spd**. We developed a mouse cDNA probe for G1r-1 (Materials and Methods) and used it to genotype backcross progeny at G1r-1 (Fig. 5). No recombinants were observed between spd and G1r-1 in 143 chromosomes examined, indicating that the two genes are located within 2.1 cM (95% confidence interval) of each other. Northern blots performed on whole brain RNA from spasmotic mice and control (C57BL/6J × C3HeB/FeJ)F1 mice demonstrated that the steady-state levels of G1r-1 transcripts were normal (Fig. 6). In addition, normal G1r-1 transcripts of approximately 5.0, 3.9, and 2.7 kb were present in the spasmotic RNA (Fig. 6) (Hollmann et al., 1989). Because the alternatively spliced forms of G1r-1 are known to be functionally different (Sommer et al., 1990), a physiologically significant change in cell-specific expression might be undetectable by Northern blot. We examined the distribution of G1r-1 mRNA in brain of spasmotic mice and littermate controls by in situ hybridization (Fig. 7). The expression of both isoforms of G1r-1 mRNA was identical in spd/spd and normal mice, indicating that cell-specific expression and alternate splicing of G1r-1 are normal in spasmotic mice.

G1r-1 cDNA was cloned from spasmotic whole brain RNA, sequenced, and compared to the cDNA sequences of rat and human (GenBank Accession Nos. M36418 and M64752, respectively) and selected portions of our control F1 mouse. The predicted amino acid sequence of the mouse cDNA was identical to the rat sequence in all but three amino acids. Amino acid 257 is an alanine in the rat and a serine residue in the mouse. This conservative difference is not significant because the human GLR1 cDNA predicts a serine in this position (Puckett et al., 1991). The other two differences between the spasmotic mouse and rat G1r-1 cDNA sequences were amino acid 843 (tryptophan in the mouse and arginine in rats and humans) and amino acid 853 (serine in the mouse and glycine in rats and humans). These two differences represent species variation because we also observed them in the cDNA from (C57BL/6J × C3HeB/FeJ)F1 mice. Several glutamate receptor mRNAs undergo RNA editing within the second transmembrane region, resulting in alteration of receptor properties (Sommer et al., 1991). G1r-1 mRNA is not normally edited, and we found no evidence for editing in the G1r-1 cDNA of spasmotic mice. The 3' untranslated region of G1r-1 transcripts varies between approximately 80 and over 1000 nucleotides. The first 42 nucleotides of the 3' untranslated region were examined and the spasmotic and F1 control mouse sequences were identical. The rat G1r-1 cDNA

**FIG. 6.** Northern blots demonstrate the presence of Anx-6 and G1r-1 transcripts in the brains of spasmotic mice. Whole brain poly(A)+ RNA was analyzed by Northern blotting. Filters from gels containing 1 and 3 μg of RNA were hybridized with cDNA probes for Anx-6 and G1r-1, respectively. The locations of 288 and 188 RNA are indicated on the left. Lanes 1 and 3, B6C3He-a/s-spd/spd; lanes 2 and 4: (C57BL6/J × C3HeB/J)F1 hybrids.

**FIG. 7.** The cellular distribution of alternatively spliced G1r-1 isoforms is normal in spd/spd mouse brain. G1r-1 is an α-amino-3-hydroxy-5-methyl-4-isoxazolidone propionic acid (AMPA)-type glutamate receptor. Two functionally different isoforms, flip and flop, generated by alternative splicing of two distinct 115-bp exons, are differentially expressed in well-defined regions of the brain (Sommer et al., 1991). The expression and distribution of two alternatively spliced forms of G1r-1, flip (A and B) and flop (C and D), were analyzed by in situ hybridization. All hybridizations were performed on midsagittal brain sections in two separate experiments, with the same result. (A, C) Normal littermate control; (B, D) spd/spd mouse.
clone contains a 5' untranslated region of approximately 197 nucleotides, with out-of-frame ATGs located 14 and 43 nucleotides upstream of the consensus AUG. The mouse Glr-1 5' untranslated region also contained these additional ATGs, and the sequences 168 bp upstream of the consensus ATG were identical in spasmodic and F1 control mice. Thus, the Glr-1 cDNA from spasmodic mice has no significant differences in the coding region or 5' untranslated region.

**DISCUSSION**

Mapping spd relative to other genes known to be on Chr 11 allowed us to rule out three promising neurotransmitter candidate genes. Adra-1 was eliminated after direct observation of multiple crossover events between it and spd. Gabra-1 and Gabrg-2 map proximal to Pad-1 and were eliminated by our localization of spd distal to Pad-1.

We report the first localization of Anx-6 and Glr-1 in mice. Both genes mapped within 2.1 cm of spd on MMU 11. Since the human counterparts to Anx-6 and Glr-1 have been localized to chromosome 5q32–q34 (Devies et al., 1989) and 5q33 (Puckett et al., 1991), our mapping extends the known synteny homology between human chromosome 5q and mouse Chr 11 (Buckwalter et al., 1991, 1992). These loci were considered as candidates for spd; however, we did not detect any evidence for rearrangement or deletion of Anx-6 or Glr-1 in spasmodic mice by Southern blot analysis of genomic DNA (data not shown). In spasmodic mouse brains, the abundance and molecular weight of Anx-6 and Glr-1 mRNA transcripts were normal, and the two alternatively expressed forms of Glr-1 exhibited appropriate cell-specific expression. Sequence analysis revealed no significant changes in the coding region or 5' untranslated region of either Anx-6 or Glr-1 cDNAs. While functional analysis of the proteins has not been carried out, the lack of significant nucleotide changes in the cDNA sequences suggests that alteration of Glr-1 or Anx-6 function in spasmodic mice is unlikely. Altered transcriptional regulation has not been directly examined but seems unlikely in view of the normal steady-state levels of both transcripts. However, subtle differences in regulation of gene expression in the mutant cannot be ruled out. The normal size of the transcripts indicates that the lesion in spd mice does not result from a gross rearrangement of Anx-6 or Glr-1 or from errors in RNA splicing or polyadenylation. Thus, there is no evidence for mutations in either Glr-1 or Anx-6 in spasmodic mice.

It is not unique to have a number of candidate genes closely linked to a mutant locus. For example, the familial adenomatosis polyposis (FAP) gene was identified after several candidate genes, including the MCC gene, were analyzed. The sex-determining gene Sry was uncovered after evidence accumulated against the neighboring candidate Zfy. In some cases clustering of related genes may reflect evolutionary history and/or shared regulatory elements, as shown for the clustered hemoglobin and homeobox gene families. While Anx-6 and Glr-1 are both members of large gene families, all members of these two families that have been mapped to date are unlinked. Nevertheless, the mutation in spasmodic mice may be in an uncharacterized annexin or glutamate receptor gene.

Five loci that cosegregated with spd were considered poor candidate genes for the mutation and were not pursued further. Although some cytokines play a role in neuronal differentiation (Hall et al., 1992), the main function of II-3, II-4, II-5, and Csfgm is believed to be in hematopoiesis and the immune system, neither of which has been reported to be altered in spasmodic mice. Sparc codes for osteonectin, a high-affinity calcium binding protein component of the extracellular matrix (reviewed in Sage et al., 1991). We consider it unlikely that Sparc is mutated in spasmodic mice because Sparc expression predominates in nonneuronal tissues (Holland et al., 1987). Higher resolution genetic analysis may provide further support for elimination of the linked genes as candidates for spd.

Spasmodic mice provide a defined genetic model for neurological syndromes featuring an abnormal startle response. The underlying lesion in abnormal startle syndromes appears to alter the amplitude of the initial reflex response to sensory stimuli and the subsequent rate of stimulus habituation. Interactions between these two components may explain why the central pathways involved in modifying the startle and related motor reflex responses extend beyond the primary sensory relay nuclei to involve limbic, brain stem reticular, cerebellar, and other descending spinal pathways (Davis, 1989; Thompson, 1988). The associated tremor and impaired righting reflex in spasmodic mice both point toward involvement of spino–cerebellar or cerebello–thalamic pathways, suggesting possible impairment of polysynaptic cerebellar outflow pathways in the brain stem. These pathways are essential for modulating adaptive gain changes in motor reflex circuits (Thompson, 1988). Pathological startle can be modulated by selective pharmacological manipulation of signaling by multiple neurotransmitters, including glutamate, gaba, noradrenaline, serotonin, dopamine, and selected peptides (Ebert et al., 1992; Liang et al., 1992; Snodgrass, 1989). The CNS anesthetic ketamine used to potentiate the startle response in the spd/spd mutants is a noncompetitive inhibitor of excitatory glutamatergic synaptic transmission at the NMDA receptor site and has been shown to facilitate the startle response (Falls et al., 1992; Mansbach et al., 1991).

The diversity of these experimental phenotypes at the molecular level suggests a reasonable likelihood for genetic heterogeneity among human abnormal startle syndromes. The available evidence suggests that startle epilepsy, or hyperekplexia (STHE), is a genetically homogeneous startle disorder (Ryan et al., 1992b). Several phenotypic characteristics of STHE suggest that the spd mutant locus provides a promising mouse model for developmental analysis of STHE. First, the absence of
EEG abnormalities, as found clinically (Ryan et al., 1992a), suggests that the lesion is subcortical, while the absence of weakness or muscular wasting excludes the involvement of lower motor neuron pathways. Second, flexion hypertonia was evident in the hindlimbs, as described in the extremities of young affected members of the human pedigree, although this diminished with age. The symptoms of STHE can be effectively treated with clonazepam (Ryan et al., 1992a), which, like AOOA, acts in part by stimulation of the gabaergic neurotransmitter system (Greenblatt et al., 1987). Thus, STHE and spd are similar in several aspects of phenotype and pharmacology.

Genetic studies provide the most convincing evidence for spasmodic mice as a model for STHE. STHE has recently been mapped to a 5-cM region of human chromosome 5, which is proximal to GABRA1 and GABRG2, but includes SPARC, GLR1, RPS14, and CSFIR (Ryan et al., 1992b). The mouse homologues of these genes map to two small regions (0-2 cM), one on mouse chromosome 18 (Rps14 and Csfrn) and the other on mouse chromosome 11 (SpArc and Glr-1). The demonstration that spd, like STHE, is linked to Gabra-1, Gabrg-2, SpArc, and Glr-1 suggests that spd is the mouse counterpart to STHE. Although we cannot exclude the possibility that the mouse counterpart to STHE is located on chromosome 18, there are currently no mutants on chromosome 18 with abnormal startle response. STHE is inherited as an autosomal dominant trait and spd as a recessive trait, and there are some phenotypic differences between the affected mice and humans. However, many diseases have both dominant and recessive mutant alleles, and different lesions in the same gene can result in dramatically different phenotypes. Regardless of whether spd and STHE represent lesions in homologous genes, the similar linkages of the mutant genes suggest that ANX6 and GLR1 should be examined for mutations in humans with STHE and imply that efforts to identify the mutant genes will be complementary. The spasmodic mouse will provide further information about abnormal startle through future studies on the pharmacology of spd and identification of the mutant gene by positional cloning.

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Note added in proof. Higher resolution genetic analysis has provided support for the elimination of Anx6 and Glr-1 as candidate genes for spd. Two recombinants were observed between spd and Glr-1 in a total of 313 individuals examined, and one recombinant was noted between spd and Anx6 in 407 individuals tested (L. M. Scarlett and N. M. Tokars).

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