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The NMDA receptor subunit 2B locus (*Nmdar2b*) maps to the distal end of murine Chromosome 6

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The N-methyl-D-aspartate (NMDA) receptor channel has been shown to be involved in long-term potentiation (LTP), an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning (Collingridge and Bliss 1987). Functional NMDA receptors appear to be composed (minimally) of an invariant subunit (R1) and one of several variable subunits (R21 through R2D; Meguro et al. 1992; Monyer et al. 1992). Each of the variable subunits exhibits a distinct distribution in the adult brain (Kutsuada et al. 1992; Meguro et al. 1992; Monyer et al. 1992). Postnatally, NMDA receptors containing the R2B subunit are primarily localized to the hippocampus and forebrain (Watanabe et al. 1992). Discerning the properties of this NMDA receptor isotype may, therefore, aid our understanding of the mechanisms of long-term potentiation.

In order to positionally map the location of the R2B subunit to the mouse genetic map, two separate probes derived from adjacent locations in the 5′ region of the NMDAr2b gene (311 and 389 bp respectively) were used. These probes gave identical results for the data presented below. A series of restriction digests of genomic C57BL/6J and DBA/2J DNA were performed. Southern analysis of these digests revealed two informative RFLPs between these lines for the *Nmdar2b* subunit locus. Specifically, use of the 389-bp probe following digestion of C57BL/6J and DBA/2J with *Pst*I gave rise to hybridizing bands of 9 and 13 kb respectively, while use of the 311-bp probe following digestion with *Bgl*II gave rise to bands of 6.5 and 5.5 kb. In each case samples were electrophoresed on a 0.7% agarose gel and transferred to Hybond N^+ nylon membranes as previously described (Ausubel et al. 1993). Blots were then hybridized overnight at 65°C with the probes and washed sequentially for 30 min in 2× SSC, and 2× SSC, 0.1% SDS at 65°C, followed by autoradiography. The enzymes *Pst*I and *Bgl*II were then used to analyze a series of 26 C57BL/6J × DBA/2J (BXD) recombinant inbred (RI) lines. These enzymes gave identical strain distribution patterns (SDPs) for R2B. These results are shown in Table 1. As can be seen from these data, the R2B distribution pattern demonstrates strong concordance with markers in the distal region of murine Chromosome (Chr) 6 and exhibits a 100% identity in its SDP with the previously described anonymous DNA marker *D6Byu1* (Woodward et al. 1992). Lines 3, 4, 7, 10, 17, and 26 were extinct at the time of these analyses, and consequently their SDPs could not be determined. The probability of linkage between *Nmdar2b* and locus *Ly-49* or marker *D6Byu1* is 1.00. From these results, *Nmdar2b* lies at a distance of 3.5 cM on either side of *D6Byu1* or 6.2 cM on either side of locus *Ly-49*, at a confidence level of 95%. The recombination frequency values for *D6Byu1/R2B* and *Ly-49/R2B* are 0 and 1.1, respectively. Linkage between *Nmdar2b* and two other loci in this region, namely *Prp* and *Rua*, decreases slightly, with values of 0.999 and 0.983 respectively. With respect to *Nmdar2b*, the SDP data for nearby loci are consistent with those previously obtained by a variety of other methods

Table 1. Strain distribution pattern of *Nmdar2b* and nearby loci.

BXD Strain:	1 2	5 6	8 9	11	16	18	25	27	32
Loci/marker									
Rho	DB	DB	BD	DBDBDB		**B**DBBBBDD		BBDB**BB**	
Qui	DB	DB	BD	DBUU**BB**		**B**DUUBUDD		BBDB**B**D	
Ly-49	DB	DB	BD	DBDBDB		**B**DBBBBDD		BBDBUU	
Prp	DB	DB	BD	DBDBDB		**B**DBBBBDD		BBDB**B**D	
Rua	DB	DB	BD	DBUUDB		**B**DUUBUDD		BBDB**B**D	
D6Byu1	DB	DB	BD	DBDBDB		DDBBBBDD		BBDBDD	
Nmdar2b	DB	DB	BD	DBDBDB		DDBBBBDD		BBDBDD	
Glb	DB	DB	DU	DBUUDB		**B**DUUBUDD		BBDB**B**D	
Xmv-24	DB	DB	DD	**B**BDBDB		DDBBB**DB**D		**D**BDBDD	

B, C57BL/6J distribution pattern. D, DBA/2J distribution pattern. U, indefined/extinct at time of determination. **Bold**, deviation from *Nmdar2b* SDP.

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(Hilliard et al. 1993) and suggests a gene order for R2B of: *Cen–Rho–Ly-49–Nmdar2b–Glb–Xmv-24*. It should be noted that the region surrounding *Ly-49* has been shown to be syntenic to human 12p (Lyon and Kirby 1993).

On the basis of the strain distribution patterns, the minimal region in which the gene for *Nmdar2b* most probably lies is defined by the Rua/R2B and R2B/Glb recombination sites present in BXD RI lines 18 and/or 31. As this is the first NMDA variable subunit to be mapped, it will be interesting to determine the position of the other variable family members, as well as the *Nmdar1*.

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