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Mice with Targeted Genetic Reduction of GABA_A Receptor $\alpha 1$ Subunits Display Performance Differences in Morris Water Maze Tasks

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Abstract

Recent research has begun to demonstrate that specific subunits of GABA_A receptors may be involved in the normal expression of specific behaviors. The present research used mice with GABA_A receptors whose $\alpha 1$ subunits contained mutations of serine 270 to histidine and leucine 277 to alanine in the TM2 region. The purpose was an attempt to examine the possible role that this particular subunit may have in learning the spatial and nonspatial version of the Morris water maze task. Mutant animals, compared to controls, displayed elevated levels of pool circling in both the spatial task and the nonspatial task. These results suggested that normal performance of the spatial and nonspatial water maze tasks may be dependent upon a natural $\alpha 1$ subunit array.

Gamma aminobutyric acid (GABA) is the primary inhibitory, as well as the most abundant, neurotransmitter in the mammalian central nervous system, with GABA receptors being estimated to be found in 30% of central nervous system neurons (Sieghart & Sperk, 2002, Morrow, 1995). It is through the ionotropic, ligand-gated GABA_A receptors that GABA exerts most of its inhibitory effects (Sieghart & Sperk, 2002). These effects include the modulation of hippocampal theta rhythms (Sun, Zhao, Nelson, & Alkon, 2001), anxiety (Liberzon, Phan, Khan, & Abelson, 2003), learning and memory (Izquierdo & Medina, 1991; Paulsen & Moser, 1998), and fast inhibitory postsynaptic potentials in hippocampal pyramidal cells that are mediated by GABA_B receptors (Lopantsev & Schwartzkroin, 1999).

GABA_A receptors are heteropentameric protein complexes whose compositions are drawn from a family of subunits, some of which contain several isoforms ($\alpha 1-6$, $\beta 1-4$, $\gamma 1-3$, $\delta 1$, $\epsilon 1$, $\theta 1$, $\pi 1$, and $\rho 1-3$) (Sieghart & Sperk, 2002; Liberzon et al., 2003). Despite the vast amount of possible subunit isoform combinations, there appears to be only a limited number of actual, *in vivo* combinations in the mammalian brain, the most common arrangement being one consisting of two $\alpha 1$ s, two $\beta 2$ s, and one $\gamma 2$ (Sieghart, 1995; McKernan & Whiting, 1996; Sieghart & Sperk, 2002). Interestingly, particular GABA_A receptor subunit combinations have been shown to be responsible for specific drug recognition and effect mediation (e.g.,

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benzodiazepines and certain anesthetics) (Morrow, 1995; Sieghart, 1995; Johnston, 1996; Sigel & Buhr, 1997; Sieghart & Sperk, 2002; Wafford et al., 2004; Sonner et al., 2005).

In trying to understand the neurological mechanisms underlying interactions of drug compounds with GABA_A receptors, the use of genetically altered animals (e.g., knockins, knockouts, reductions, etc.) coupled with behavioral tasks has proven valuable. A logical line of thought that arises is what roles, if any, do specific GABA_A receptor subunits have in the expression of certain overt behaviors. An emerging line of research has begun to demonstrate such links between particular GABA_A receptor subunits and behavioral tasks. Recently, lines of mice have been created that possess amino acid mutations in specific transmembrane (TM) regions of GABA_A receptor $\alpha 1$ subunits. An initial "knockin" mouse with a serine 270 to histidine mutation in the TM2 region of the $\alpha 1$ subunit displayed a variety of phenotypic alterations, including an increased sensitivity to GABA (Nishikawa, Jenkins, Paraskevakis, & Harrison, 2002), particular behavioral alterations, and prolonged decay of mIPSCs in hippocampal neurons (Homanics et al., 2005). A second $\alpha 1$ "knockin" animal with both serine 270 to histidine and leucine 277 to alanine mutations in the TM2 region resulted in GABA_A receptors with near normal GABA sensitivity but insensitivity to volatile anesthetics (Werner et al, 2006; Borghese et al, 2006; Sonner et al., 2007). This knockin animal recorded selective alterations in hippocampal mIPSCs indicating potentially altered hippocampal function, yet contextual fear conditioning was not affected in these animals (Sonner et al., 2007).

Mice with the aforementioned, latter GABA_A receptor $\alpha 1$ subunit mutations (Werner et al, 2006; Borghese et al, 2006; Sonner et al., 2007) were used in the present study to investigate a possible behavioral role of the $\alpha 1$ subunit in the standard spatial and nonspatial versions of the Morris water maze task. Specifically, control mice were homozygous for serine at 270 and leucine at 277 while knockin mice (hereafter known as "mutants") were homozygous for histidine at 270 and alanine at 277 (Borghese, et al., 2006; Werner, et al., 2006). All subject mice were male, littermate offspring that were task-naïve. The results suggested that normal performance in both the spatial and nonspatial tasks might be dependent upon GABA_A receptors comprised of natural $\alpha 1$ subunits.

Spatial training occurred over nine days. Two probe trials were administered the day following spatial training completion, each having a ceiling time of 45 seconds and originating from the maze's north starting position. The first probe trial saw the submerged escape platform removed, forcing the animal to search for the escape platform for the duration of the trial. The second probe trial, which was conducted to verify that the escape platform was not visible during spatial training, saw the submerged escape platform moved to the quadrant opposite the location used during spatial training. Nonspatial training initiated two days following the completion of probe trials and lasted for five days (see Berry & Matthews, 2004 for a detailed description of spatial and nonspatial training). Water temperature was kept constant at 72.8° F.

Results from the spatial task illustrated that mutant animals were displaying performance differences during spatial training. First, mutant animals swam significantly longer path lengths on Training Days 4, 6, and 7 (two-way ANOVA with repeated measures, $F(8, 144) = 2.092$, $p = 0.005$; see Figure 1). Further analysis revealed that on Training Days 4 and 6, mutant animals displayed higher levels of pool circling than control animals (two-way ANOVA with repeated measures, $F(8, 144) = 2.876$, $p = 0.005$; see Figure 2). These results could not be explained by floating or swim speed scores as there were no differences in floating between groups (two-way ANOVA with repeated measures, $F(1, 18) = 2.999$, $p = 0.1$) and control animals swam consistently faster than knockins (two-way ANOVA with repeated measures, $F(1, 18) = 5.189$, $p = 0.035$). Therefore, it appeared that the longer path lengths by mutant animals were the result of employing this looping, rotational search strategy more often than

control animals. Pool circling, however, could not explain the higher path length scores on Training Day 7. This suggests that mutant animals may have either switched to yet another search strategy that was beyond the recognition of the tracking system and the investigators, or it may have suggested that they were employing a search strategy that was similar to controls but were, at this point in the training procedure, displaying a lower level of acquired spatial knowledge than that acquired by the control animals.

Mutant animals also displayed lower Gallagher Global Proximity scores (two-way ANOVA with repeated measures, $F(1, 18) = 11.921, p = 0.003$), a measure that provides an animal's average distance from the escape platform during their trials (i.e., a "homing in" on the escape platform). Normally, a search pattern should get tighter, or closer, to the escape platform as training progresses and learning occurs. However, mutant animals presented search patterns that were consistently farther away from the escape platform compared to controls, meaning their search patterns were not closing in on the escape platform location as well as controls.

An alternative explanation for these specific differences could be that mutant animals were exhibiting heightened anxiety on these particular days. A recent study demonstrated that these particular mutant mice do not show heightened anxiety on an elevated plus maze (Werner et al, 2006). However, an analysis of thigmotaxis, a particular measure that has been suggested to correspond to open field anxiety, revealed a strong trend toward the possibility of elevated anxiety in mutant animals (two-way ANOVA with repeated measures, $F(1, 18) = 4.238, p = 0.054$).

Mutant animals also displayed differences during probe trials, suggesting the possibility that learning differences had occurred during spatial training. During the first probe trial, mutant animals swam less of their path length in the escape platform quadrant than did controls (two-way ANOVA with repeated measures, $F(3, 54) = 4.53, p = 0.023$). In addition, mutant animals made fewer passes across the escape platform location than did controls (two-way ANOVA with repeated measures, $F(3, 54) = 4.42, p = 0.026$) and displayed a search pattern that was farther away from the escape platform location than controls (one-way ANOVA, $F(1, 18) = 6.03, p = 0.024$).

During the second probe trial, mutant animals, compared to controls, swam less of their path lengths in the quadrant where the escape platform was originally located while, at the same time, swimming a greater path length percentage in the quadrant where the escape platform had been moved (two-way ANOVA with repeated measures, $F(3, 54) = 3.56, p = 0.036$). Interestingly, this was not due to the mutant animals acquiring the escape platform more often as both groups found the escape platform with equivalent frequency [$\chi^2(1, n = 20) = 0.067, p = 0.795$]. This seemed to indicate that mutant animals were displaying a less likely tendency to proceed to the original escape platform location before acquiring the escape platform in its new location.

Surprisingly, mutant animals displayed a similar performance difference in the nonspatial task. While mutant and control animals learned to acquire the escape platform with an equivalent frequency (two-way ANOVA with repeated measures, $F(1, 16) = 3.951, p = 0.064$), mutant animals did so by swimming longer path lengths (two-way ANOVA with repeated measures, $F(1, 16) = 14.859, p = 0.001$; see Figure 1 insert). Again, this pattern appeared to be the result of increased pool circling as mutant animals were employing this particular search strategy more often than control animals across training days (two-way ANOVA with repeated measures, $F(1, 16) = 5.619, p = 0.031$; see Figure 2 insert). These results could not be explained by elevated anxiety as thigmotaxis results revealed no difference between controls and mutant animals (two-way ANOVA with repeated measures, $F(1, 16) = 1.883, p = 0.189$). Neither could they be explained by floating and swim speed scores as there was no difference in floating

between groups (two-way ANOVA with repeated measures, $F(1, 16) = 3.124$, $p < 0.10$) and controls swam consistently faster than knockins (two-way ANOVA with repeated measures, $F(1, 16) = 8.877$, $p = 0.009$). To address the possibility of a carryover effect of pool circling from the spatial task to the nonspatial task, an analysis of the four nonspatial Training Day 1 trials was conducted. A carryover effect may in fact be present as there was a strong trend, but no significant difference, between mutant and control animals on Training Day 1 (two-way ANOVA with repeated measures, genotype by trial, $F(1, 16) = 3.803$, $p = 0.069$).

Because of the similar performance differences in both the spatial and nonspatial tasks, there was concern that mutant animals may have possessed an abnormal morphology of the eye. Structural examination revealed no morphologic differences between groups at the level of the retina and fundus. Visual system deficits beyond the level of the eye, however, could not be ruled out.

In summary, it was demonstrated that mice with the aforementioned GABA_A receptor $\alpha 1$ subunit mutations displayed similar performance differences in both the spatial and nonspatial Morris water maze tasks which appeared to be the result of an increased utilization of a circling type of search strategy. Overall, it appears that GABA_A receptor $\alpha 1$ subunits may play an arbitrating role in the performance of the spatial and nonspatial versions of this task in mice with this particular background strain. In interpreting the present results, the authors acknowledge the small sample size and the fact that only male mice were used. Consequently, further work is needed. Future studies should also investigate the possibility of hippocampal and/or visual system impairment, as well as the possibility of motivational differences, as explanations for these findings.

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References

- Berry RB, Matthews DB. Acute ethanol administration selectively impairs spatial memory in C57BL/6J mice. *Alcohol* 2004;32:9–18. [PubMed: 15066698]
- Borghese CM, Werner DF, Topf N, Baron NV, Henderson LA, Boehm SL II, et al. An isoflurane- and alcohol-insensitive mutant GABA_A receptor $\alpha 1$ subunit with near-normal heterologous systems and production of knockin mice. *The Journal of Pharmacology and Experimental Therapeutics* 2006;319(1):208–218. [PubMed: 16807363]
- Homanics GE, Elsen FP, Ying S-W, Jenkins A, Ferguson C, Sloat B, et al. A gain-of-function mutation in the GABA_A receptor produces synaptic and behavioral abnormalities in the mouse. *Genes, Brain, and Behavior* 2005;4:10–19.
- Izquierdo I, Medina JH. GABA_A receptor modulation of memory: The role of endogenous benzodiazepines. *Trends in Pharmacological Sciences* 1991;12:260–265. [PubMed: 1658996]
- Johnston GAR. GABA_A receptor pharmacology. *Pharmacology & Therapeutics* 1996;69(3):173–198. [PubMed: 8783370]
- Liberzon I, Phan KL, Khan S, Abelson JL. Role of the GABA_A receptor in anxiety: evidence from animal models, molecular and clinical psychopharmacology, and brain imaging studies. *Current Neuropharmacology* 2003;1:267–283.
- Lopantsev V, Schwartzkroin PA. GABA_A-dependent chloride influx modulates GABA_B-mediated IPSPs in hippocampal pyramidal cells. *The Journal of Neurophysiology* 1999;82(3):1218–1223.
- McKernan RM, Whiting PJ. Which GABA_A-receptor subtypes really occur in the brain? *Trends in Neurosciences* 1996;19(4):139–143. [PubMed: 8658597]
- Morrow AL. Regulation of GABA_A receptor function and gene expression in the central nervous system. *International Review of Neurobiology* 1995;38:1–41. [PubMed: 8537199]

- Nishikawa K, Jenkins A, Paraskevakis I, Harrison NL. Volatile anesthetic actions on the GABA_A receptors: contrasting effects of alpha 1(S270) and beta 2(N265) point mutations. *Neuropharmacology* 2002;42:337–345. [PubMed: 11897112]
- Paulsen O, Moser EI. A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends in Neurosciences* 1998;21:273–278. [PubMed: 9683315]
- Sieghart W. Structure and pharmacology of γ -aminobutyric acid_A receptor subtypes. *Pharmacological Reviews* 1995;47(2):181–234. [PubMed: 7568326]
- Sieghart W, Sperk G. Subunit composition, distribution and function of GABA_A receptor subtypes. *Current Topics in Medicinal Chemistry* 2002;2:795–816. [PubMed: 12171572]
- Sigel E, Buhr A. The benzodiazepine binding site of GABA_A receptors. *Trends in Pharmacological Sciences* 1997;18:425–429. [PubMed: 9426470]
- Sonner JM, Cascio M, Xing Y, Fanselow MS, Kralic JE, Morrow AL, et al. $\alpha 1$ subunit-containing GABA type A receptors in forebrain contribute to the effect of inhaled anesthetics on conditioned fear. *Molecular Pharmacology* 2005;68:61–68. [PubMed: 15833735]
- Sonner JM, Werner DF, Elsen FP, Xing Y, Liao M, Harris RA, et al. Effect of isoflurane and other potent inhaled anesthetics on minimum alveolar concentration, learning, and the righting reflex in mice engineered to express $\alpha 1$ γ -aminobutyric acid type A receptors unresponsive to isoflurane. *Anesthesiology* 2007;106(1):107–113. [PubMed: 17197852]
- Sun M, Zhao W, Nelson T, Alkon DL. Theta rhythm of hippocampal CA1 neuron activity: gating by GABAergic synaptic depolarization. *The Journal of Neurophysiology* 2001;85(1):269–279.
- Werner FW, Blednov YA, Ariwodola OJ, Silberman Y, Logan E, Berry RB, et al. Knockin mice with ethanol-insensitive $\alpha 1$ -containing γ -aminobutyric acid type A receptors display selective alterations in behavioral responses to ethanol. *The Journal of Pharmacology and Experimental Therapeutics* 2006;319(1):219–227. [PubMed: 16785315]
- Wafford KA, Macaulay AJ, Fradley R, O'Meara GF, Reynolds DS, Rosahl TW. Differentiating the role of γ -aminobutyric acid type A (GABA_A) receptor subtypes. *Biochemical Society Transactions* 2004;32:553–556. [PubMed: 15157182]

Figure 1. Spatial Training

Mean path length values were measured across training days in the Morris water maze spatial task. A * indicates a significant mean difference between genotypes. Error bars represent S.E.M.

Figure 2. Nonspatial Learning

Mean path length values were measured across training days in the Morris water maze nonspatial task. Error bars represent S.E.M.