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Brief Report

Mice with targeted genetic reduction of $GABA_A$ receptor α1 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 α1 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 α1 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 (Morrow, 1995; Sieghart & Sperk, 2002). 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 (α_{1-6}, β_{1-4}, γ_{1-3}, δ_1, ε_1, θ_1, π_1, and ρ_{1-3}) (Liberzon et al., 2003; Sieghart & Sperk, 2002). 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 α1s, two β2s, and one γ2 (McKernan & Whiting, 1996; Sieghart, 1995; 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., benzodiazepines and certain anesthetics) (Johnston, 1996; Morrow, 1995; Sieghart, 1995; Sieghart & Sperk, 2002; Sigel & Buhr, 1997; Sonner et al., 2005; Wafford et al., 2004).

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 α1 subunits. An initial "knockin" mouse with a serine 270 to histidine mutation in the TM2 region of the α1 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 α1 "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 (Borghese et al., 2006; Sonner et al., 2007; Werner et al., 2006). This knockin animal recorded selective

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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 α1 subunit mutations (Borghese et al., 2006; Sonner et al., 2007; Werner et al., 2006) were used in the present study to investigate a possible behavioral role of the α1 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 α1 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 s 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, $F(8,144) = 2.092$, $p = .005$; see Fig. 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, $F(8,144) = 2.876$, $p = .005$; see Fig. 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, $F(1,18) = 2.999$, $p = .1$) and control animals swam consistently faster than knockins (two-way ANOVA with repeated measures, $F(1,18) = 5.189$, $p = .035$). 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, $F(1,18) = 11.921$, $p = .003$), 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, $F(1,18) = 4.238$, $p = .054$).

Mutant animals also displayed differences during probe trials, suggesting the possibility that learning differences had occurred

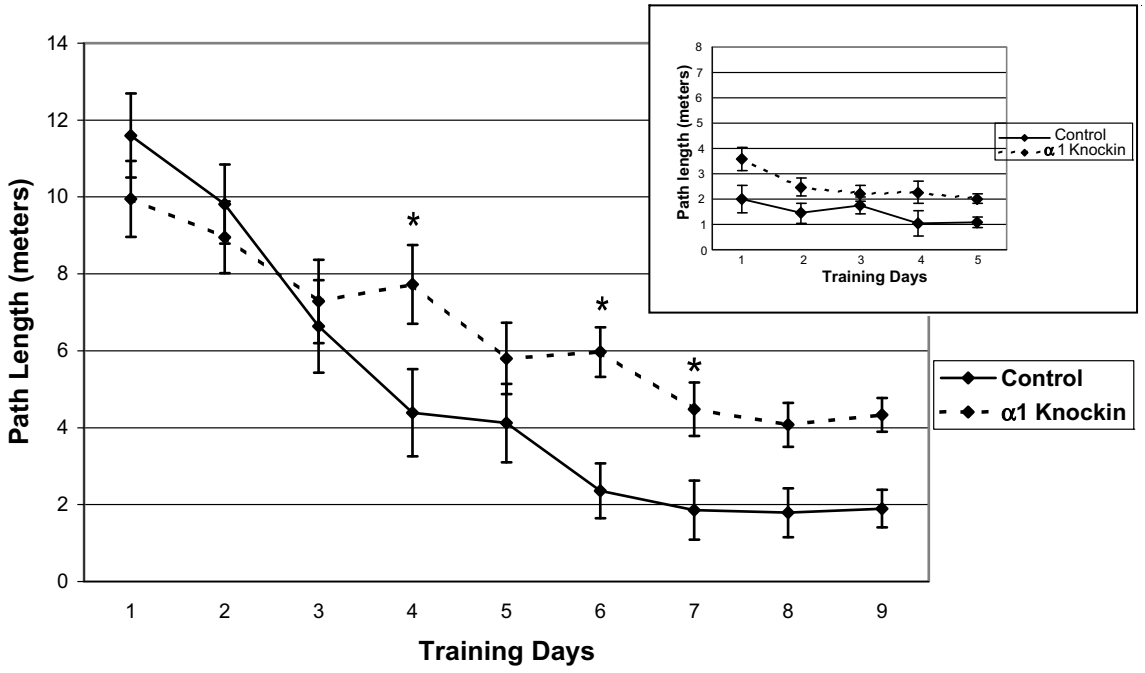

Fig. 1. Spatial training. Mean path length values were measured across training days in the Morris water maze spatial task. (Insert) Spatial training. Mean pool circling values were measured across training days in the Morris water maze spatial task. A * indicates a significant mean difference between genotypes. Error bars represent S.E.M.