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Trace and contextual fear conditioning is enhanced in mice lacking the α4 subunit of the $GABA_A$ receptor

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ABSTRACT

The $GABA_AR$ α4 subunit is highly expressed in the dentate gyrus region of the hippocampus at predominantly extra synaptic locations where, along with the $GABA_AR$ δ subunit, it forms $GABA_A$ receptors that mediate a tonic inhibitory current. The present study was designed to test hippocampus-dependent and hippocampus-independent learning and memory in $GABA_AR$ α4 subunit-deficient mice using trace and delay fear conditioning, respectively. Mice were of a mixed C57Bl/6J X 129S1/X1 genetic background from α4 heterozygous breeding pairs. The α4-knockout mice showed enhanced trace and contextual fear conditioning consistent with an enhancement of hippocampus-dependent learning and memory. These enhancements were sex-dependent, similar to previous studies in $GABA_AR$ δ knockout mice, but differences were present in both males and females. The convergent findings between α4 and δ knockout mice suggests that tonic inhibition mediated by α4βδ $GABA_A$ receptors negatively modulates learning and memory processes and provides further evidence that tonic inhibition makes important functional contributions to learning and behavior.

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1. Introduction

The γ-aminobutyric acid A receptor ($GABA_AR$) functions as the major mediator of both phasic and tonic inhibitory neurotransmission in the brain. It is composed of five subunits selected from 19 gene products that are currently known to play a role in the mammalian CNS (α1–6, β1–3, γ1–3, δ, ε, π, θ, and ρ1–3) (Olsen & Sieghart, 2008). Receptors containing α4βδ are predominantly expressed extrasynaptically where they mediate a tonic inhibitory current (Glykys, Mann, & Mody, 2008) that is sensitive to modulation by low doses of neurosteroids and ethanol (Brown, Kerby, Bonnert, Whiting, & Wafford, 2002; Smith, Shen, Gong, & Zhou, 2007; Wallner, Hanchar, & Olsen, 2003). The α4 subunit is primarily expressed in the molecular layer of the dentate gyrus, thalamus, striatum, nucleus accumbens, tuberculum olfactorium, and superficial layers of the neocortex, while being notably absent from the cerebellum (Cestari, Liu, Mu, & Burt, 1998; Chandra et al., 2006; Pirker, Schwarzer, Wieselthaler, Sieghart, & Sperk, 2000). Although typically expressed together, the α4 and δ subunits can partner with other subunits. δ can combine with α6 in the cerebellar granule cells and α1 in inhibitory interneurons in the molecular layer of the dentate gyrus (Glykys et al., 2007; Pirker et al., 2000; Sperk, Schwarzer, Tsunashima, Fuchs, & Sieghart, 1997), whereas α4 can combine with γ2 to form sub-synaptic $GABA_AR$'s (Benke, Michel, & Mohler, 1997; Sur et al., 1999).

Electrophysiological studies have found that $GABA_AR$ α4 KO mice show greatly reduced tonic inhibitory currents and slower mIPSC kinetics in dentate gyrus granule cells and thalamic relay neurons (Chandra et al., 2006; Liang et al., 2008). $GABA_AR$-mediated inhibition plays a critical role in modulating the dynamics of the neural circuitry thought to underlie learning and memory processes, influencing both information processing and synaptic plasticity (Buzsaki, 1997; Crabtree & Isaac, 2002; Paulsen & Moser, 1998; Read, Nenov, & Halgren, 1994). The goal of the present study was to determine the learning and memory phenotype of $GABA_AR$ α4 KO mice utilizing Pavlovian tone fear conditioning.

In tone fear conditioning an initially neutral tone conditional stimulus (CS) is paired with an aversive shock unconditional stimulus (US). In delay conditioning, the shock is temporally contiguous with the tone whereas in trace conditioning the shock onset follows a "trace" interval of 20 s after tone offset. Delay tone conditioning results from strengthening of the thalamic and cortical inputs which

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convey auditory information to sites of CS and US convergence within the amygdala (Boatman & Kim, 2006). Trace conditioning, however, requires the additional contribution of the hippocampus in order to support formation of the CS–US association (Chowdhury, Quinn, & Fanselow, 2005; McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; Misane et al., 2005; Quinn, Loya, Ma, & Fanselow, 2005; Quinn, Oommen, Morrison, & Fanselow, 2002; Tseng, Guan, Disterhoft, & Weiss, 2004). Trace and delay fear conditioning, therefore, allow for the assessment of hippocampus-dependent and hippocampus-independent learning, respectively. A previous study found that $GABA_AR$ δ KO mice exhibit normal delay fear conditioning but enhanced trace and contextual fear conditioning, with this behavioral effect limited to female mice (Wiltgen, Sanders, Ferguson, Homanics, & Fanselow, 2005). This indicated that loss of the δ-subunit produces a specific sex-dependent enhancement in hippocampus-dependent learning and memory. This sex difference was hypothesized to be due to fluctuating neurosteroid levels in the female mice and the action of these endogenous compounds on δ-containing $GABA_ARs$, specifically in the dentate gyrus (Wiltgen et al., 2005). The present study, utilizing identical experimental procedures, assessed trace and delay fear conditioning in male and female $GABA_AR$ α4 KO mice.

2. Materials and methods

All mice were produced in Pittsburgh (Chandra et al., 2006), shipped to UCLA and allowed a 4–6 week acclimation time. Mice ranged from 4–7 months of age at the time of study. They were of a mixed C57Bl/6J X 129S1/X1 genetic background from α4 heterozygous breeding pairs. The $GABA_AR$ α4 KO mice were compared to their WT littermates. Mice were genotyped as described earlier (Chandra et al., 2006). All were housed in the Psychology Department vivarium at UCLA on a 12 h light/12 h dark cycle in groups of 3–5 and had free access to food and water. Experiments were performed on both female and male mice during the light phase of the cycle (n = 8–10 per group, for a total of 74 mice).

For a more detailed description of the methods see (Wiltgen et al., 2005). As outlined in Fig. 1, training took place in four identical chambers (Context A) (28 × 21 × 21 cm; Lafayette Instrument Co.) in a brightly lit room. The floor of each chamber consisted of a shock grid wired to a shock generator and scrambler (Med-Associates Inc.) to deliver foot shock. On the training day (Day 1) α4 KO or WT mice underwent either trace or delay fear conditioning. For both procedures, mice were placed in Context A and left to explore for 3 min before tone onset (20 s, 75 dB, 2800 Hz). For delay conditioning, termination of tone was contiguous with foot shock (2 s, 0.5 mA). For trace conditioning, tone and foot shock were separated by a 20-s trace interval. Both groups received five tone-shock trials. In order to keep the overall session length the same, the trace conditioned group received a 200-s ITI (inter-trial interval, defined as the time between CS onset and the next CS onset) and the delay conditioned group received a 220-s ITI. After the last shock, animals were left for 2 min and then returned to their home cages.

For the tone test (Day 2), 24 h after training, the animals were placed in a separate room with four novel and structurally distinct chambers (Context B). The tone was delivered in the same way as in Context A during training, but with shock omitted. On Day 3, 24 h after the tone test, animals were returned to the holding room for 30 min before being placed back in Context A for an 8 min context test.

An observer blind to the genotype of the animal scored the presence or absence of freezing as a measure of conditional fear. For the tone test, freezing scores were grouped into three different bins: baseline (BL), tone, and post-tone or "trace" interval (the 20 s immediately following tone termination). For the BL bin, freezing was scored every 8 s. For both tone and trace bins, freezing was scored every 2 s. Freezing data from the five tone presentations were averaged together. During the context test on Day 3, freezing was scored every 8 s. All freezing scores were transformed into percent freezing by dividing the total number of freezing by the total number of observations and multiplying by 100 (%Freezing = $Freeze_{TOT}/Observations_{TOT} \times 100$). The data was analyzed using the general linear model statistical package SPSS (SPSS Inc.) with α set at 0.05.

3. Results

The results indicate that $GABA_AR$ α4 subunit deletion tended to enhance Pavlovian fear conditioning but the precise effects depend on the sex of the animal and the type of conditioning. Data from both trace and delay conditioned animals were combined and a 3-way ANOVA, with the factors genotype (WT vs. KO), training (trace vs. delay), and sex (male vs. female), was performed for baseline, tone, trace, and context freezing. Data for the tone test for delay conditioned animals are shown in Fig. 2, data for the traced conditioned animals are shown in Fig. 3, and the results of the context test are shown in Fig. 4. Female mice froze significantly more than male mice during both the tone and post-tone intervals, as well as the context test, regardless of training or genotype ($F_{(1,73)}$ = 7.909, p = 0.006; $F_{(1,73)}$ = 7.023, p = 0.010; and $F_{(1,74)}$ = 6.498, p = 0.013 respectively). As expected, there was an overall enhancement in delay conditioned relative to trace conditioned mice during the tone presentation ($F_{(1,73)}$ = 20.828, p < 0.001). $GABA_AR$ α4 KO mice showed a significant enhancement of freezing during the tone ($F_{(1,73)}$ = 11.616, p = 0.001), trace ($F_{(1,73)}$ = 4.515, p = 0.037), and context ($F_{(1,74)}$ = 5.437, p = 0.023) tests. Trace conditioned $GABA_AR$ α4 KO mice froze significantly more during the tone ($F_{(1,36)}$ = 7.994, p = 0.008), but delay conditioned $GABA_AR$ α4 KO mice did not differ ($F_{(1,37)}$ = 3.233,

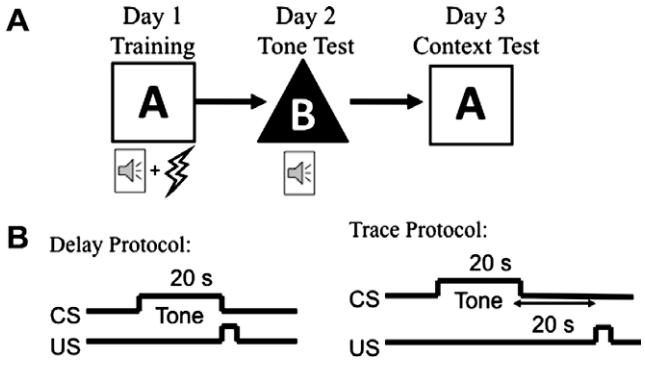

Fig. 1. Experimental Design. (A) Outline of 3 day procedure. (B) Diagram depicting temporal relationship between tone and shock in delay vs. trace conditioning.

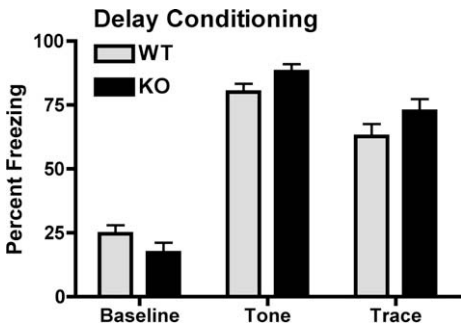

Fig. 2. $GABA_AR$ α4 KO mice show no differences in delay tone fear conditioning. Mean (± SEM) percent freezing of α4 KO and WT mice during the BL, tone, and trace intervals of the tone test for delay conditioned animals.