Hippocampal α5 subunit-containing GABA_A receptors are involved in the development of the latent inhibition effect

T.V. Gerdjikov a,1, U. Rudolph b,d, R. Keist b, H. Möhler b,c, J. Feldon a, B.K. Yee a,*

a Laboratory of Behavioral Neurobiology, ETH Zurich, Schwerzenbach, Switzerland
b Institute of Pharmacology and Toxicology, University of Zurich, Zurich, Switzerland
c Institute of Pharmaceutical Sciences, ETH Zurich and Collegium Helveticum, Zurich, Switzerland
d Laboratory of Genetic Neuropharmacology, McLean Hospital, and Department of Psychiatry, Harvard Medical School, Belmont, Massachusetts, USA

Received 22 March 2007; revised 5 June 2007; accepted 6 June 2007
Available online 16 July 2007

Abstract

Hippocampal GABA_A receptors containing the α5 subunit have been implicated in the modulation of hippocampal-dependent learning, presumably via their tonic inhibitory influence on hippocampal glutamatergic activity. Here, we examined the expression of latent inhibition (LI)—a form of selective learning that is sensitive to a number of manipulations targeted at the hippocampal formation, in α5(H105R) mutant mice with reduced levels of hippocampal α5-containing GABA_A receptors. A single pre-exposure to the taste conditioned stimulus (CS) prior to the pairing of the same CS with LiCl-induced nausea was effective in reducing the conditioned aversion against the taste CS in wild-type mice—thus constituting the LI effect. LI was however distinctly absent in male α5(H105R) mutant mice. Hence, a partial loss of hippocampal α5 GABA_A receptors is sufficient to alter one major form of selective learning, albeit this was not seen in the female. This observed phenotype suggests that specific activation of these extrasynaptic GABA_A receptors may confer therapeutic potential against the failure to show selectivity in learning by human psychotic patients.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Attention; Benzodiazepine; Conditioned taste aversion; GABA; Learning; Schizophrenia

1. Introduction

GABAAergic activity can critically modulate normal hippocampal functions, presumably via their inhibitory influence on hippocampal glutamatergic neurotransmission. A specific subset of hippocampal GABA_A receptors, distinguished by the presence of the α5 subunit are located extrasynaptically on pyramidal cells and mediate tonic inhibition. The functional relevance of hippocampal α5 GABA_A receptors includes the regulation of a variety of hippocampal-related behaviours. This has been demonstrated in knock-in mice bearing a point mutation (H105R) in the GABA_A receptor α5 subunit gene, which is associated with a reduced expression of α5 subunit-containing GABA_A receptors restricted to hippocampal subfields CA1 and CA3 (Crestani et al., 2002). In the α5(H105R) mice, the distribution and level of expression of the mutated α5 GABA_A receptor was unaltered in all brain areas except in hippocampal pyramidal cells, which displayed a striking dendritic receptor deficit (see Crestani et al., 2002). In the hippocampus, α5 GABA_A receptors are largely extrasynaptic both in wild-type and α5(H105R) mice, although outside the hippocampus α5 GABA_A receptors are also present in synaptic locations. They are likely to contribute to the regulation of dendritic excitability of hippocampal pyramidal cells and the efficacy of excitatory inputs, especially during simultaneous firing of interneurons when GABA spills over to adjacent extrasynaptic GABA_A receptors (Galarreta & Hestrin, 1999; Gibson, Beierlein, & Connors, 1999). The α5(H105R) mice appar-
ently displayed normal LTP as shown in an in vitro slice preparation (see Crestani et al., 2002), although alternative protocols for LTP evaluations may be warranted (e.g., with in vivo preparation) to ascertain the integrity of this form of synaptic plasticity.

The behavioural phenotypes of these knock-in mice included mild elevation in spontaneous open field activity, impaired sensorimotor gating (Hauser et al., 2005), improved conditioning under trace condition in which CS and US are discontiguous or temporally separated (Crestani et al., 2002; Yee et al., 2004), and resistance to extinction of learned response (Yee et al., 2004). The spectrum of behavioural abnormalities reported matches the modulatory functions of the hippocampus over response selection, including the acquisition and expression of learned behaviour (e.g., Bast & Feldon, 2003; Bangasser, Waxler, Santollo, & Shors, 2006; Beylin et al., 2001; Douglas, 1967; Jarrard, 1968; Jarrard, Feldon, Rawlins, Sinden, & Gray, 1986; Koch, 1996; Rawlins, Feldon, & Gray, 1980; Solomon, Vander Schaaf, Thompson, & Weisz, 1986).

One suggestion is that hippocampus modulates these behaviours via its interaction with the mesolimbic dopaminergic system at the nucleus accumbens (Sesack & Pickel, 1990; Totterdell & Meredith, 1997; Totterdell & Smith, 1989). Spontaneous locomotor activity (Beninger, 1983), the trace conditioning effect (Norman & Cassaday, 2003), sensorimotor gating in the form of prepulse inhibition (PPI) (Ellenbroek, Budde, & Cools, 1996; Mansbach, Geyer, & Braff, 1988), and resistance to extinction (Weiner, Bercowitz, Lubow, & Feldon, 1985) can be sensitive to dopamine agonist treatment. Selective learning in the form of latent inhibition (LI) (Lubow & Moore, 1959) is another phenomenon, which is also under the control of mesolimbic dopamine (Ellenbroek et al., 1996) and the modulation by the hippocampal system (see Weiner, 1990; Weiner, 2003; Yee, Feldon, & Rawlins, 1995). LI refers to the observation that prior non-reinforced pre-exposures of the conditioned stimulus (CS) impede the generation of a conditioned response (CR) to the CS when the CS is subsequently paired with an unconditioned stimulus (US). LI expression is blocked by amphetamine treatment (Weiner, Lubow, & Feldon, 1988; Norman and Cassaday 2003) have recently shown that the trace conditioning effect is similarly affected by amphetamine. In both cases, amphetamine enhances conditioned responding under conditions in which control subjects show a weak response: either induced by CS pre-exposure or CS–US discontinuity. Moreover, both effects are known to be sensitive to the damage to the hippocampal system (Bangasser et al., 2006; Beylin et al., 2001; Honey & Good, 1993; Moyer, Deyo, & Disterhoft, 1990; Oswald et al., 2002; Yee et al., 1995).

Regarding these parallel actions of amphetamine, it is interesting to note that DeViti, Bauste, Nett, and Barrett (1987) have speculated that LI may be mediated by the trace conditioning effect. Accordingly, CS pre-exposures preferentially reduce attention to later segments of individual CS, which then effectively act as a trace interval between initial CS segments and the US during subsequent CS–US pairings; the perceived discontinuity thereby weakens conditioning and yields the LI effect (also see Ayres, Albert, & Bombace, 1987). Hence, animals insensitive to the trace conditioning effect may be predicted to be insensitive to the LI (i.e., CS pre-exposure) procedure. α5(H105R) mice show better acquisition of associative learning than wild-type (wt) mice in the trace conditioning but not the delayed conditioning procedure (Crestani et al., 2002), and hence the trace conditioning effect is attenuated in the α5(H105R) mice (Yee et al., 2004). It may therefore be hypothesized that the expression of LI would also be attenuated in these mice.

Here, this hypothesis was tested in a conditioned taste aversion LI paradigm. We assessed the efficacy of a single pre-exposure to the taste CS, one day prior to taste → gastric malaise pairing, in reducing the subsequent expression of learned aversion towards the taste CS in α5(H105R) male and female mice in comparison to wild-type controls.

2. Materials and methods

2.1. Subjects

The α5(H105R) mutation was generated in RW-4 embryonic stem cells derived from 129X1/SvJ mice and maintained on a 129X1/SvJ inbred background (RCC, Füllinsdorf, Switzerland) as described by Crestani et al. (2002). The subjects were bred in our laboratory’s in-house specific pathogen free facility (ETH Zurich Laboratory of Behavioural Neurobiology, Schwerzenbach, Switzerland). Both male and female mice were used, with 22 α5(H105R) mice and 23 wild-type controls. Individual group sizes in each experimental condition are listed in Table 1. Mice were weaned and separated by gender at postnatal day 21. At age 8–10 weeks mice were transferred to a separate vivarium for experimental animals (22 ± 2°C; humidity, 55 ± 5%) and caged singly. The animals were maintained on a 12:12 h reversed light–dark cycle (lights on at 2000 h), with ad libitum food (Kliba 3430, Klibamühlen, Kaiseraugst, Switzerland). Water was freely available until the start of the water restriction protocol.

2.2. Apparatus

The experiment was conducted in polycarbonate home cages measuring 16 × 22 cm in floor space and 14 cm in height (Techniplast, SA, Milan, Italy) with sawdust bedding, in which the animals were kept throughout the experiment. Each cage was equipped with two drinking tubes made from 15 ml polypropylene test tubes (Cellstar®, Greiner Bio-One, Frickenhausen, Germany) and equipped with an air-tight screw top. An opening of 2.5 mm in diameter was made at the end of each tube, thus allowing the animal access to the liquid without any leakage. The two drinking tubes were secured by two acrylic rings (20 mm in inner diameter) attached to the slant surface of the cage’s grid top, thus allowing the efficient placement and removal of the drinking tubes. When in place, the drinking tips were 35 mm apart and 50 mm above the cage floor, enabling the animals to switch easily between tubes and to sample both liquids.

2.3. Procedure

Testing was conducted daily between 1000 and 1800 h in the dark phase of the circadian cycle. Water deprivation was gradually introduced over a 6-day period, with daily access to water reduced from 8, 4, and 2 h in the first three days, and to 1 h in the last three days. The experiment began on the next day as described below:
Counterbalanced.

Pre-exposed (nPE), with their baseline drinking performance and sex were subdivided into two subgroups: pre-exposed (PE) and non-pre-exposed (nPE), with their baseline drinking performance counterbalanced.

Pre-exposure: On day 5, PE and nPE subjects were given access to 10% sucrose solution and normal drinking water, respectively, in both drinking tubes during the first drinking period. All animals received water in the second drinking period on this day.

Conditioning: On day 6, all animals received 10% sucrose solution in both drinking tubes in the first drinking period, and followed immediately by an intraperitoneal injection of LiCl solution (0.25 M, 2% of body weight), which induced gastric nausea and served as the US. All animals received water in the second drinking period on this day.

Test: On day 7, conditioned taste aversion was assessed using a "two-bottle" procedure in the first drinking period, in which each animal was given access to one drinking tube containing water, and the other sucrose solution. Aversion to the taste CS was indexed by the relative consumption between sucrose solution and water. Reduced aversion in the PE subgroup was seen across days and between sexes. On the 4th day of baseline, the amount of water consumed in the first drinking period per group was (mean ± SEM): $\alpha5(\text{H105R})$ male = 1.6 ± 0.1 g, $\alpha5(\text{H105R})$ female = 1.5 ± 0.1 g, wt male = 1.9 ± 0.1 g, wt female = 1.7 ± 0.1 g. Animals of each group were then subdivided into PE and nPE subgroups with matching baseline drinking performance.

### 3.2. Pre-exposure

On this day, nPE animals consumed water and PE animals consumed sucrose solution in the first drinking period. The data are summarised in Table 1. $\alpha5(\text{H105R})$ mice consumed less liquid than wt mice regardless of sex [$F(1,37) = 5.93, p < .05$]. There was no difference between sexes, or between PE and nPE conditions.

### 3.3. Conditioning

On this day, all animals consumed sucrose solution in the first drinking before being injected with the nausea-inducing agent, LiCl. The liquid consumption data are summarised in Table 1. All animals exhibited sickness behaviour following injection of LiCl, and this was largely indistinguishable between mutant and control mice, including a marked reduction in water consumption on the second drinking period on this day. The analysis of liquid consumption in the first drinking period only yielded a significant pre-exposure effect [$F(1,37) = 5.09, p < .05$], because PE subjects consumed less liquid than nPE subjects, regardless of sex or genotype.

### 3.4. Test

Analysis of total liquid consumed (sucrose and water combined) on this day did not yield any significant main

---

**Table 1**

Mean (±SEM) amount of liquid (g) consumed during the 30 min pre-exposure and conditioning sessions for male and female $\alpha5(\text{H105R})$ or wt mice

<table>
<thead>
<tr>
<th>Group size</th>
<th>Pre-exposure</th>
<th>Conditioning</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nPE (water)</td>
<td>PE (sucrose)</td>
<td>nPE (total)</td>
</tr>
<tr>
<td>$\alpha5(\text{H105R})$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta$</td>
<td>6 ± 0.29</td>
<td>1.87 ± 0.31</td>
<td>2.63 ± 0.48</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>5 ± 0.34</td>
<td>1.90 ± 0.34</td>
<td>2.89 ± 0.59</td>
</tr>
<tr>
<td>wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta$</td>
<td>2.24 ± 0.33</td>
<td>2.75 ± 0.35</td>
<td>3.20 ± 0.16</td>
</tr>
<tr>
<td>$\varphi$</td>
<td>2.22 ± 0.26</td>
<td>2.40 ± 0.28</td>
<td>2.88 ± 0.27</td>
</tr>
</tbody>
</table>

The content of the solution is given in parentheses for each pre-exposure condition, expect for the test day of which the total (sucrose + water) liquid consumed is provided.

**Baseline:** On days 1–4, water was available for two 30-min drinking sessions at 1200 and at 1600 h, with water available at both periods. Water intake was stabilised over the 4-day period. The animals of each genotype and sex were subdivided into two subgroups: pre-exposed (PE) and non-pre-exposed (nPE), with their baseline drinking performance counterbalanced.

**Conditioning:** On day 6, all animals received 10% sucrose solution in both drinking tubes in the first drinking period, and followed immediately by an intraperitoneal injection of LiCl solution (0.25 M, 2% of body weight), which induced gastric nausea and served as the US. All animals received water in the second drinking period on this day.

**Test:** On day 7, conditioned taste aversion was assessed using a “two-bottle” procedure in the first drinking period, in which each animal was given access to one drinking tube containing water, and the other sucrose solution. Aversion to the taste CS was indexed by the relative consumption between sucrose solution and water. Reduced aversion in the PE subgroup was seen across days and between sexes. On the 4th day of baseline, the amount of water consumed in the first drinking period per group was (mean ± SEM): $\alpha5(\text{H105R})$ male = 1.6 ± 0.1 g, $\alpha5(\text{H105R})$ female = 1.5 ± 0.1 g, wt male = 1.9 ± 0.1 g, wt female = 1.7 ± 0.1 g. Animals of each group were then subdivided into PE and nPE subgroups with matching baseline drinking performance.

2.4. Data analysis

Liquid consumption was measured by subtracting the weight of each drinking tube after each drinking period from its weight taken at the start of the period, and expressed in grams. Data from the four phases of the experiment were separately analysed.

Baseline daily water consumption in the first drinking period was subjected to a $2 \times 2 \times 4$ (sex $\times$ genotype $\times$ days) split-plot analysis of variance (ANOVA). Liquid consumptions (the first drinking period) on the pre-exposure and conditioning days were analysed by separate 2 (sex) $\times$ 2 (genotype) ANOVA. The total liquid consumption and sucrose consumption on the test day were also similarly analysed. In addition, sucrose solution consumption on the test day was expressed as percentage of total liquid consumption: 100% $\times$ sucrose/(sucrose + water consumption), and subjected to a $2 \times 2 \times 2$ (sex $\times$ genotype $\times$ pre-exposure) ANOVA. Significant interactions were further evaluated by Fisher LSD pair-wise comparisons to ascertain the pattern of the interaction.

All statistical analyses were conducted using SPSS for Windows™ (version 13.0) implemented on a PC running the Windows XP Professional SP2 operating system (Microsoft).

3. Results

3.1. Baseline

There was a gradual reduction of water consumption in the first drinking period across the four baseline days

$[F(3, 123) = 87.23, p < .001]$ as the subjects were acclimatized to 2 periods per day drinking schedule. Overall, water consumption was higher in male mice [$F(1, 41) = 21.47, p < .001$], but this sex difference disappeared by the end of the baseline phase, leading to the emergence of a significant interaction between sex and days [$F(3, 123) = 3.83, p < .01$]. Mutant mice consumed less water in general [$F(1, 41) = 8.20, p < .01$], and this effect was consistently seen across days and between sexes. On the 4th day of baseline, the amount of water consumed in the first drinking period per group was (mean ± SEM): $\alpha5(\text{H105R})$ male = 1.6 ± 0.1 g, $\alpha5(\text{H105R})$ female = 1.5 ± 0.1 g, wt male = 1.9 ± 0.1 g, wt female = 1.7 ± 0.1 g. Animals of each group were then subdivided into PE and nPE subgroups with matching baseline drinking performance.

3.2. Pre-exposure

On this day, nPE animals consumed water and PE animals consumed sucrose solution in the first drinking period. The data are summarised in Table 1. $\alpha5(\text{H105R})$ mice consumed less liquid than wt mice regardless of sex [$F(1,37) = 5.93, p < .05$]. There was no difference between sexes, or between PE and nPE conditions.

3.3. Conditioning

On this day, all animals consumed sucrose solution in the first drinking before being injected with the nausea-inducing agent, LiCl. The liquid consumption data are summarised in Table 1. All animals exhibited sickness behaviour following injection of LiCl, and this was largely indistinguishable between mutant and control mice, including a marked reduction in water consumption on the second drinking period on this day. The analysis of liquid consumption in the first drinking period only yielded a significant pre-exposure effect [$F(1,37) = 5.09, p < .05$], because PE subjects consumed less liquid than nPE subjects, regardless of sex or genotype.

3.4. Test

Analysis of total liquid consumed (sucrose and water combined) on this day did not yield any significant main
effects or interaction terms. Test for conditioned aversion to the sucrose solution was assessed by the sucrose solution consumed in proportion to total liquid consumption. Increasing aversion is associated with a reduction in this measure (see Fig. 1).

LI refers to reduced conditioned aversion in the PE relative to the nPE group. As illustrated in Fig. 1, this difference was detected in all four (2 sexes × 2 genotypes) groups, yielding an overall significant main effect of pre-exposure \( F(1,37) = 26.3, p < .001 \). Notably, the comparison between male \( \alpha 5(105R) \) and wild-type mice (Fig. 1a) suggested an attenuation of the CS pre-exposure effect (i.e., the LI effect) in the mutant, whilst the expression of LI was not affected by the mutation in female mice (Fig. 1b). It therefore appears that the \( \alpha 5(105R) \) mutation attenuated LI in male but not female mice. The 3-way interaction (genotype × pre-exposure × sex) approached statistical significance \( F(1,37) = 3.89, p = .056 \).

This pattern of results is accompanied by a tendency of weaker LI in the female wild-type mice relative the male wild-type mice. This impression was supported by a 2-way ANOVA restricted to the wild-type mice which yielded a near-significant interaction between pre-exposure and sex \( F(1,19)=4.32, p = .05 \). Given the differential expression of LI between sexes in the wild-type mice, we in addition conducted separate analysis to evaluate the effect of the \( \alpha 5(105R) \) mutation in each of the two sexes. A significant interaction between genotype and pre-exposure was obtained in the analysis restricted to the male \( F(1,19)=6.41, p = .02 \), but not in the female \( F<1 \). The analysis restricted to the female only yielded a significant pre-exposure effect \( F(1,18)=7.92, p = .01 \). Supplementary Fisher’s LSD pair-wise comparisons confirmed that the interaction term in the male was associated with (i) the presence of a statistically significant LI effect in the wild-type controls \( [p < .01] \) but not \( \alpha 5(105R) \) mice \( [p = .71] \), and (ii) the loss of LI in male \( \alpha 5(105R) \) mice was attributable to a reduction of the CS pre-exposure effect in the \( \alpha 5(105R) \) mice relative to the wild-type control \( \alpha 5(105R) \) PE vs wild-type PE \( p < .05 \).

4. Discussion

The present study represents the first attempt to investigate the role of any specific GABA\_A receptor subtype, as characterized by its constituent \( \alpha \) subunit, in the development of latent inhibition (LI)—a form of selective learning (Lubow, 1989). Here, we demonstrated that the \( \alpha 5(105R) \) mutation, which reduces expression of extrasynaptically located \( \alpha 5 \) subunit-containing GABA\_A receptors in the hippocampus, attenuates the expression of LI in male mice without affecting conditioned taste aversion as such because performance in the nPE condition was not affected. The lack of an effect in the nPE condition is consistent with previous reports showing that aversive as well as appetitive simple delayed classical conditioning remains largely unaffected by the \( \alpha 5(105R) \) mutation (Crestani et al., 2002; Yee et al., 2004). This is also consistent with the finding that, although local activation of hippocampal GABA\_A receptors by muscimol can affect the development of the conditioned response in the Pavlovian conditioned freezing paradigm, its effect was mainly seen in contextual conditioning but not in conditioning to a discrete CS (Bast, Zhang, & Feldon, 2001; Holt & Maren, 1999). The abolition of LI seen here was therefore specifically and entirely attributed to a loss of the CS pre-exposure effect; non-reinforced pre-exposure to the CS failed to affect subsequent CS–US associative learning in the male mutant. Given that the mutation is associated with a partial deletion of \( \alpha 5 \) subunit-containing GABA\_A receptors in the hippocampus (Crestani et al., 2002), our results suggest that inhibitory activity normally mediated by this receptor subtype may be involved in the modulation and development of the LI effect. This is in keeping with the recent finding that intra-hippocampal stimulation of GABA\_A receptors by muscimol administration enhances the LI effect in the same conditioning paradigm employed here (Stone, Grimes, & Katz, 2005). Our finding is in general agreement with the hypothesis that the hippocampal system plays a crucial role in the modulation of LI expression (Gray, Feldon, Rawlins, Hemsley, & Smith, 1991; Weiner, 1990; Weiner, 2003; Weiner & Feldon, 1997), and shows that \( \alpha 5 \) GABA\_A receptor-mediated GABA\_ergic neurotransmission in the hippocampus participates in this modulation. It is noteworthy that several lesion experiments have shown that damage to either the medial septum (Turgeon et al., 2001) or the hippocampus (Purves, Bonardi, & Hall, 1995; Reilly, Harley, & Revusky, 1993) is associated with an enhancement of LI of conditioned taste aversion, although it is not clear at the moment if the opposite effects observed following these lesions are necessarily in agreement with the highly selective molecular intervention.
employed here. Consideration for the possibility that our the α5(H105R) mutation might affect the functional connection (directly or indirectly via interneurons) between the medial septum and the hippocampus is warranted.

While this particular phenotype was only demonstrated in the male, both male and female α5(H105R) mutant mice showed reduced liquid consumption during the baseline phase. This effect persisted in the pre-exposure session (see Table 1), but disappeared from the conditioning phase onwards. First, this shows that the mutation was not behaviourally ineffective in the female mice here. Second, because this effect was equivalently seen in both sexes of the mutant, it is unlikely that the observed LI disruption in male mice stemmed purely from the reduction in the consumption of sucrose solution during pre-exposure—i.e., develops as a result of reduced CS pre-exposure. In fact, with the present paradigm of CTA latent inhibition, we typically do not find any correlation in the PE subjects between the amounts of sucrose solution consumed (with the exclusion of non-drinkers) on the pre-exposure day and the magnitude of conditioned aversion developed subsequent to conditioning.

Here, a clear effect of the α5(H105R) mutation was observed only in the male mice, which contrasted with the lack of such an effect in the female mice. Should one conclude from it that the mutation that had led to a partial deletion of the α5 GABA_A receptors in both sexes was exclusively effective in inducing the observed phenotype in the male sex? First, it should be noted that while the clear effect in the male is supported statistically by the presence of a genotype by pre-exposure interaction, the 3-way interaction (genotype × pre-exposure × sex) failed to attain statistical significance. Just as the lack of a significant genotype × pre-exposure interaction in the female dose not constitute statistical support for the null hypothesis, neither does the lack of a significant 3-way interaction (see Abelson, 1995). Additional evidence needs to be sought elsewhere.

Although there was little evidence in general for a sex difference in the expression of LI in normal subjects, including humans (e.g., Lubow et al., 2001; Maes, 2002; Swerdlow et al., 2005), and that in females the expression is largely independent of the menstrual cycle (Swerdlow et al., 2005), a number of manipulations, especially those involving early neurodevelopmental manipulation (hence subjects of both sexes are typically included), have been reported to preferentially disrupt LI in the male. In particular, early-life non-handling (Peters, Gray, & Joseph, 1991; Shalev, Feldon, & Weiner, 1998; Weiner et al., 1985; Weiner, Feldon, & Ziv-Harris, 1987) and prenatal stress (Bethus, Lemaire, Lhomme, & Goodall, 2005; Shalev & Weiner, 2001) have been reported by several groups to disrupt LI in male but not in female rats. A similar sex difference is also reported following corticosterone administration (Shalev & Weiner, 2001). Hence, there is some evidence for the claim that male subjects might be more sensitive to manipulations disrupting LI (e.g., Bethus, Lemaire, Lhomme, & Goodall, 2005), and this would fit our present observation.

On the other hand, it is not entirely impossible that the same trait can be uncovered in the female mutant with changes in paradigm or test parameters. Here, the magnitude of LI in the control wild-type female appeared weaker than in the male controls. The latter might have facilitated the detection of the LI-disruption phenotype in the male. One possibility is to include a higher number of pre-exposures (e.g., extended to 3–5 days), and this may enable the detection of a similar phenotype in the female. Another possibility is to adopt a different associative learning paradigm, as it is not unprecedented that a manipulation can be effective in disrupting LI in one but not another paradigm. We have previously reported that lesions of the nucleus accumbens shell failed to disrupt LI in the conditioned taste aversion paradigm but the same lesions led to a complete abolition of LI in the active 2-way avoidance procedure (e.g., Pothuizen, Jongen-Realo, Feldon, & Yee, 2006). A switch of paradigm would be recommended before a firm conclusion as to whether the α5(H105R) mutation’s effect on LI is specific to the male sex alone.

This caution is warranted by our previous experience with this particular mutant mouse line. The α5(H105R) mutation is also known to facilitate learning under trace conditioning (Pavlov, 1927), i.e., they are less sensitive to a trace delay inserted between CS and US presentation that normally weakens the development of a conditioned response (Crestani et al., 2002). In the female, this phenotype was only demonstrable in an aversive fear conditioning paradigm, while in the male, the same effect can only be seen in an appetitive conditioning paradigm (Yee et al., 2005). Hence, mutant mice of both sexes share the same phenotypic trait, even though the optimal conditions conducive to its expression may differ between sexes. Additional reasons for caution are observations of two additional behavioural phenotypes of the α5(H105R) mutation that are independent of sex, namely prepulse inhibition impairment (Hauser et al., 2005) and a resistance to extinction of the conditioned freezing response (Yee et al., 2004).

Taken together the α5(H105R) mutation exerts multiple effects on Pavlovian associative learning but only in conditions in which conditioned responding is normally weak in control animals as a result of specific parametric manipulations in accordance to learning theories. Thus, α5(H105R) mutant showed stronger conditioned responding in trace conditioning, during extinction, and in latent inhibition. They are, respectively, less able to modulate their acquisition and/or expression of the conditioned response in accordance to CS-US discontiguity (i.e., the trace conditioning effect), and to non-reinforced CS presentation after (i.e., in extinction) as well as before (i.e., latent inhibition) CS–US pairing. This may reflect a general deficit in adaptive associative learning in the mutant mice, perhaps stemming from a hyperactive mesolimbic dopaminergic system as alluded to in the Introduction. However, it seems improbable that these diverse effects can be readily subsumed under a single psychological trait. The trace condi-
tioning effect is generally explained in terms of overshadowing by relative temporal proximity between CS and context (see Mackintosh, 1983); extinction is believed to involve some forms of inhibitory conditioning processes (Davis & Myers, 2002); and LI is typically attributable to learning to ignore, to tune out, or to reduce attention to stimuli that appear irrelevant in the prediction of reinforcement (e.g., Lubow, 1989; Mackintosh, 1973). Despite the interesting attempt by DeVietti et al. (1987) to explain LI in terms of trace conditioning, their account still relies on selective attention developed as a consequence of CS pre-exposure—a process that is not required in the explanation of the trace conditioning effect. Alternative accounts of LI also point to the relevance of priming by context (e.g., Wagner, 1976; Wagner, 1978), and to proactive interference whereby a memory of the [CS → no consequence] relationship experienced by subjects during pre-exposure interferes with the retrieval of an effective [CS → US] association acquired during subsequent conditioning (Bouton, 1993; Kraemer & Spear, 1991). Interestingly, the presence of LI attenuation and increased resistance to extinction would be conflicting within the explanatory framework of the proactive interference account of LI (also see Weiner, 1990). Additional experiments using various selective learning paradigms including, for instance, blocking (Kamin, 1990) and relative cue validity (Wagner, 1969) would be highly warranted. Not only would such experiments enable a more comprehensive characterization of the cognitive phenotypes present in this mutant mouse line, but they would also allow the identification of the potential variety of cognitive processes sensitive to hippocampal GABAergic modulation via α5 subunit-containing GABA_A receptors.

An attenuation of hippocampal local GABAergic neurotransmission may enhance the excitability of hippocampal principal neurons (Freund & Antal, 1988; Freund & Buzsaki, 1996). Activation of the ventral hippocampus induced by intracerebral NMDA infusion is known to attenuate LI in rats (Pouzet, Zhang, Weiner, Feldon, & Yee, 2004). Moreover, the same manipulation in rats also disrupts PPI (Zhang, Bast, & Feldon, 2002), which matches another phenotype of the α5(H105R) mice (Hauser et al., 2005). Both traits have been suggested to reflect the positive symptoms of schizophrenia (e.g., Feldon & Weiner, 1991; Gray, Pickering, Hemsley, Dawling, & Gray, 1992; Lubow, 2005; Moser, Hitchcock, Lister, & Moran, 2000; Swerdlow, Braff, Taaid, & Geyer, 1994; Weiner, 1990). Although a recent study of trace conditioning in schizophrenia failed to find a clear effect of enhanced conditioning under the trace condition as seen in α5(H105R) mice (Crestani et al., 2002; Yee et al., 2004), the authors did observe the presence of maladaptive responding during the trace in schizophrenic patients (Marenco, Weinberger, & Schreurs, 2003). Taken together, the phenotypic profile of α5(H105R) mice reinforces our recent suggestion that disturbance of hippocampal α5 GABA_A receptor function or distribution may be implicated in the symptom genesis of schizophrenia (Hauser et al., 2005). In this regard, functional imaging in symptomatic schizophrenia patients has yielded evidence that specific clusters of symptoms, characterized by distortions of reality in the forms of delusions and hallucinations, are associated with increases in metabolic activity localized to the parahippocampal gyrus (Liddle et al., 1992). A partial loss of α5 subunit-containing GABA_A receptor mediated inhibition within this region may be expected to give rise to such dysfunctional metabolic pattern. Given the existence of direct projections from the hippocampus and entorhinal cortex to the ventral striatum (Sepask & Pickel, 1990; Totterdell & Meredith, 1997; Totterdell & Smith, 1989), the possibility that the pathogenesis may further involve a functional hyperactivity of the mesolimbic dopaminergic system developed as a consequence of altered limbic–striatal interaction would require direct evaluation by in vivo neurochemical assays.

In conclusion, the present study provides further evidence for a role of hippocampal α5 subunit-containing GABA_A receptors in the control over the selective nature of associative learning, and suggests that their loss may be involved in the disease mechanism of schizophrenia. This strengthens the suggestion that multiple GABA_A receptor subtypes are involved, via distinct neuroanatomical pathways, in the aetiology of schizophrenia with current hypotheses pointing to the relevance of cortical α2 GABA_A receptors (Lewis & Hashimoto, 2007; Lewis, Hashimoto, & Volk, 2005) as well as subcortical α3 GABA_A receptors (Yee et al., 2005). This is in line with the suggestion that specific benzodiazepines that are devoid of activity at α1 GABA_A receptors but act as full positive allosteric modulators of GABA action at α2, α3, and α5 subunit-containing GABA_A receptors may confer therapeutic efficacy for schizophrenia (Guidotti et al., 2005; Möhler, 2007).

Acknowledgments

This research was supported by ETH Zürich, the NCCR Neural Plasticity and Repair, Swiss National Science Foundation.

References


ventral hippocampal lesions, but is attenuated following local activation of the ventral hippocampus by intracerebral NMDA infusion. Neuroscience, 124, 183–194.


Sesack, S. R., & Pickel, V. M. (1990). In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in opposition to each other. Brain Research, 527, 266–279.


