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Sensorimotor Gating Deficits in Transgenic Mice Expressing a Constitutively Active Form of $G_s\alpha$

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Schizophrenia is a complex disorder characterized by wide-ranging cognitive impairments, including deficits in learning as well as sensory gating. The causes of schizophrenia are unknown, but alterations in intracellular G-protein signaling pathways are among the molecular changes documented in patients with schizophrenia. Using the CaMKIIα promoter to drive expression in neurons within the forebrain, we have developed transgenic mice that express a constitutively active form of $G_s\alpha$ ($G_s\alpha$*), the G protein that couples receptors such as the D_1 and D_5 dopamine receptors to adenylyl cyclase. We have also generated mice in which the CaMKIIα promoter drives expression of a dominant-negative form of protein kinase A, R(AB). Here, we examine startle responses and prepulse inhibition of the startle reflex (PPI) in these $G_s\alpha$* and R(AB) transgenic mice. $G_s\alpha$* transgenic mice exhibited selective deficits in PPI, without exhibiting alterations in the startle response, whereas no deficit in startle or PPI was found in the R(AB) transgenic mice. Thus, overstimulation of the cAMP/PKA pathway disrupts PPI, but the cAMP/PKA pathway may not be essential for sensorimotor gating. $G_s\alpha$* transgenic mice may provide an animal model of certain endophenotypes of schizophrenia, because of the similarities between them and patients with schizophrenia in G-protein function, hippocampus-dependent learning, and sensorimotor gating.
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INTRODUCTION

Schizophrenia is a complex and chronic illness characterized by positive psychotic symptoms as well as negative symptoms such as social withdrawal, impaired social functioning, and cognitive dysfunction (Andreasen and Black, 1995). Population and familial studies demonstrate that schizophrenia has a strong genetic component that may depend on the presence and, possibly, interaction of alleles at several different loci (for reviews, see Bassett *et al*, 2000; Sawa and Snyder, 2002). Although it is not possible to model a phenotype as complex and distinctly human as schizophrenia in animals, it is feasible to identify measurable subphenotypes, or endophenotypes, characteristic of patients with schizophrenia that can be reproduced in genetically altered laboratory rodents (Bucan and Abel, 2002; Tarantino and Bucan, 2000). This approach is useful in identifying candidate genes that may contribute to schizophrenia.

One endophenotype that has been the focus of many studies is deficient sensorimotor gating, a reduction in the brain's ability to filter excessive sensory information and generate appropriate motor responses. Aberrant gating is commonly identified in tests of prepulse inhibition of startle (PPI) (reviewed in Koch, 1999). In these tests, a startle response produced by a strong unexpected stimulus is compared to a response produced by an identical stimulus preceded ~50–150 ms by a lower intensity prestimulus. Normally, a decreased startle response is observed when the prestimulus precedes the startle stimulus. However, multiple neuropsychiatric disorders that are associated with a reduced ability to gate intrusive information are also associated with deficits in PPI (reviewed in Koch, 1999). For instance, schizophrenia patients and their first-degree relatives exhibit a smaller reduction in the startle response or, in other words, show reduced PPI (Cadenhead *et al*, 2000; Geyer and Braff, 1987; Swerdlow *et al*, 1994; Swerdlow and Geyer, 1998). In addition, investigators have also developed protocols to assess auditory gating directly in the brain by using surface electrodes or, in animals, intracranial electrodes, and have found similar results

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(Adler *et al*, 1982; Boutros *et al*, 1991; Clementz *et al*, 1997; Connolly *et al*, 2003; Erwin *et al*, 1991; Freedman *et al*, 1996; Judd *et al*, 1992; Stevens *et al*, 1996).

Schizophrenia has been proposed to result from changes in a variety of neurotransmitter systems including glutamate, dopamine, and serotonin (reviewed by Owen, 2000; Sawa and Snyder, 2002; Weickert and Kleinman, 1998). Studies in rodents have demonstrated that each of these systems has some role in regulating sensorimotor gating. In particular, dopamine and serotonin agonists and NMDA antagonists strongly disrupt PPI (reviewed in Geyer *et al*, 2001). Further dissection of the dopaminergic pathways that contribute to PPI by the use of selective dopamine receptor antagonists that have similar pharmacological actions as typical antipsychotic medications has provided additional support for a dopamine-based abnormality in schizophrenia. Specifically, dopaminergic signaling through the D_2 receptor but not D_3, D_4, or D_5 receptors is thought to be involved in PPI (Peng *et al*, 1990; Ralph *et al*, 1999). Additionally, signaling through D_1 receptors may potentiate the effect of D_2 signaling (Peng *et al*, 1990; Wan *et al*, 1996) and, in mice, D_1-family receptor agonists disrupt PPI (Holmes *et al*, 2001; Ralph-Williams *et al*, 2003). It is less clear exactly which serotonin receptors are involved in PPI. Agonist/antagonist studies suggest that the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptors may contribute (Dulawa *et al*, 1997, 1998; Rigdon and Weatherspoon, 1992; Sipes and Geyer, 1994, 1995a, b), although other members of the serotonin receptor family have yet to be investigated.

Aside from alterations to neurotransmitter receptors, mutations in signal transduction molecules downstream of these receptors could have strong effects on sensorimotor gating. One likely class of proteins in this category is the G proteins to which dopamine, serotonin, and glutamate receptors couple. Interestingly, changes to G_s, G_i, and G_o have been observed in patients with schizophrenia. Specifically, the left hemisphere of patients with schizophrenia appears to have reduced $G_i\alpha$, $G_o\alpha$, and $G_q\alpha$ immunoreactivity (Nishino *et al*, 1993; Okada *et al*, 1991, 1994; Yang *et al*, 1998). In addition, mononuclear leukocytes isolated from untreated patients have elevated levels of $G_s\alpha$ that are not observed in patients receiving clozapine or haloperidol (Avissar *et al*, 2001).

G_s-coupled receptors stimulate the cAMP/PKA pathway. Activation of this signal transduction cascade may disrupt PPI by acting similarly to D_1/D_5 receptor agonists, which disrupt PPI (Holmes *et al*, 2001; Ralph-Williams *et al*, 2003). Alternatively, activation of the cAMP/PKA signaling pathway may enhance neural transmission, thus stimulating dopamine release in the nucleus accumbens. As an initial attempt to examine the role of G proteins and the cAMP/PKA system in sensorimotor gating, we measured PPI in wild-type and transgenic mice that express a constitutively active form of $G_s\alpha$ ($G_s\alpha^*$) (Abel *et al*, 1997a; Wand *et al*, 2001) and transgenic mice that express a dominant-negative form of protein kinase A, R(AB) (Abel *et al*, 1997b). Both transgenes are under the control of the CaMKIIα promoter that drives expression in neurons within forebrain areas including the neocortex, olfactory bulb, hippocampus, striatum, and amygdala (Mayford *et al*, 1996). These transgenic mice exhibited selective impairments in hippocampus-dependent forms of memory and alterations in hippocampal synaptic physiology (Abel *et al*, 1997a, b). If $G_s\alpha^*$ animals also have a disruption of PPI without any alteration in baseline startle, this would support a hypothesis that excessive stimulation of the cAMP/PKA pathway by elevated G_s signaling (or possibly, in the context of schizophrenia, by reduced G_i signaling) produces sensorimotor gating deficits and possibly other endophenotypes of schizophrenia. The examination of PPI and startle in the R(AB) transgenic mice allows us to determine if PKA in forebrain neurons is necessary for sensorimotor gating. Portions of this research have been presented in abstract form (Gould and Abel, 2000).

MATERIAL AND METHODS

Genetically Modified Mice

$G_s\alpha^*$ and R(AB) transgenic mice were maintained in breeding colonies at the University of Pennsylvania in a hemizygous state on a C57BL/6J background. Currently, the $G_s\alpha^*$ transgenic mouse colony is at N7–N11 on a C57BL/6J background and the R(AB) transgenic mouse colony is at N8–N10 on a C57BL/6J background. R(AB) carries mutations in both cAMP-binding sites and acts as a dominant inhibitor of both types of PKA catalytic subunits (Clegg *et al*, 1987; Ginty *et al*, 1991a, b; Mellon *et al*, 1989). R(AB) transgenic mice used in this study were from the R(AB)-1 line described in Abel *et al* (1997b), and the forebrain-specific CaMKIIα promoter was used to drive the expression of this transgene. $G_s\alpha^*$ transgenic mice were from the $G_s\alpha^*$-1 transgenic mouse line. In these $G_s\alpha^*$ transgenic mice, the CaMKIIα promoter was used to drive expression of a constitutively active mutation, $G_s\alpha$Q227L (Abel *et al*, 1997a; Wand *et al*, 2001). In the Wand *et al* (2001) study, expression of the $G_s\alpha^*$ ($G_s\alpha$Q227L) was demonstrated in the hippocampus, cortex, striatum, and nucleus accumbens (see Figure 5, Wand *et al*, 2001). Consistent with our analysis of other transgenic lines, the CaMKIIα promoter drove transgene expression sufficient to increase basal adenylyl cyclase activity 2–4-fold, depending on the brain region studied (see Table 4, Wand *et al*, 2001). For genotyping, tail DNA was prepared and analyzed by Southern blotting using a transgene-specific probe as described (Abel *et al*, 1997b). Mutants and wild-type littermate controls were tested in a balanced fashion in each experiment. In most cases, within a given litter, a transgenic mouse was selected for experiments only if it could be paired with a wild-type animal of the same sex. Animals used in PPI experiments were at least 8 weeks old and group housed with *ad lib* access to food and water. Both male and female wild-type and transgenic mice were used in the experiments. In a previous study, no significant effects of sex were observed (Gould and Abel, 2000).

Startle Response and Prepulse Inhibition Tests

Reflexive startle and PPI, the ability of a low-intensity stimulus to decrease startle response, were tested using the SR-Lab System (San Diego, CA) as described in Tarantino *et al* (2000). The system includes a $35 \times 33 \times 38.5\,cm^3$ sound-attenuating chamber ventilated with a small fan and illuminated by a 15 W light bulb mounted in the ceiling. The

chambers contained a stabilimeter that consists of a clear Plexiglas cylinder ($16 \times 8.75\,cm^2$) mounted to a Plexiglas frame ($12.5 \times 20.5 \times 0.6\,cm^3$). The cylinder and frame were elevated 2.75 cm above a 30 cm × 30 cm × 4 cm Plexiglas base by four screws stationed under each corner of the stabilimeter frame. Acoustic stimuli were delivered through 6 cm speakers mounted in the enclosure ceilings, 23 cm above the cylinders. Startle responses were transduced by the piezoelectric accelerometers mounted beneath the Plexiglas frames. Output signals were digitized, rectified, and recorded as consecutive 1 ms readings on an IBM 486 computer using San Diego Instruments Startle Reflex software. The average startle response and maximum amplitude of the startle response were recorded as San Diego Instruments startle units.

Mice were tested using two different startle/PPI protocols that were based on protocols successfully used to study PPI in prior studies (Tarantino *et al*, 2000; Ralph *et al*, 1999). These programs differed in the interstimulus interval, the duration of the sampling of the startle response, whether intertrial intervals varied, and the decibel levels at which prepulses were presented. Despite these differences, the two startle/PPI protocols yielded similar results. In each case, mice were acclimated for at least 30 min in the testing facility before beginning startle/PPI sessions. Percent PPI was calculated using the following formula: {100−(startle + prepulse/startle alone) × 100}. PPI was reported as percent inhibition of the average startle response. Errors reported represent the standard error of the mean. The maximum amplitude of the startle response within the collection window was also evaluated and was shown to correlate with the average startle response (Gould and Abel, 2000; Tarantino *et al*, 2000).

Protocol 1

This protocol is based on a PPI protocol used by Tarantino *et al* (2000). Each session started with 5 min of 65 dB background noise, followed by both startle and PPI trials. Startle trials consisted of 40 ms pulses at 90, 95, 100, 105, 110, 115, and 120 dB. Each trial was presented five times in a randomized order with a 15-s intertrial interval. Startle trials were followed by PPI trials. For these trials, a 20 ms prepulse terminated 40 ms prior to the onset of a 40 ms 120 dB startle stimulus. PPI was recorded for prepulses of 73, 78, 82, and 87 dB. All prepulse trials were presented five times in a randomized order with a 15-s intertrial interval. Data were recorded as 100 1 ms voltage readings, which were averaged over the collection interval to give an average startle measure for each trial.

Protocol 2

This protocol is based on a PPI protocol used by Ralph *et al* (1999). Each session started with a 5 min acclimation period with a 65 dB acoustic background noise followed by five 120 dB startle pulses, in an effort to make subsequent startle trials less variable. Startle trials consisted of 40 ms pulses at 0 (no stimulus), 90, 95, 100, 105, 110, 115, and 120 dB. Each trial was presented five times in a randomized order with an intertrial interval randomized from 10 to 20 s. Startle trials were followed by PPI trials. Each prepulse was 20 ms in duration, followed by a 40 ms startle stimulus of 120 dB. There was a 100 ms interstimulus interval between prepulse offset and startle stimulus onset. PPI was recorded for prepulse intensities of 69, 73, and 81 dB, and no stimulus. Each prepulse trial was administered five times in a random order. Data were collected as 60 1 ms voltage readings, which were averaged over the collection interval to give an average startle measure for each trial.

Data Analysis

SPSS (SPSS, version 11.5) was used for statistical analyses described in this paper. A repeated-measures analysis of variance was used to detect differences with a minimal significance level of $\alpha = 0.05$. Stimulus level was the repeated measure and genotype was compared across subjects. Transgenic mice and the corresponding wild-type mice were compared. Tukey-adjusted *post hoc* comparisons were used to examine effects when interactions were found. For protocol 2, mice were tested in a no-stimulus condition (No Stim) to test for differences in baseline levels of activity. These data were analyzed separately, comparing transgenic to matched wild-type mice using independent *t*-tests.

RESULTS

$G_s\alpha^*$ Transgenic Mice, but not R(AB) Transgenic Mice, Exhibit PPI Deficits (Protocol 1)

Protocol 1 tested PPI with a 40 ms interstimulus interval between the 20 ms prepulse and the 40 ms startle stimulus. $G_s\alpha^*$ transgenic mice ($n = 8$) and wild-type littermate mice ($n = 8$) were tested for startle reactivity at seven different intensities and tested for PPI at four prepulse intensities, each paired with a 120 dB startle stimulus (Figure 1). The average $G_s\alpha^*$ startle response was not significantly different from wild-type mice ($F(1, 14) = 0.66$; $p = 0.81$), nor was there a significant genotype × dB interaction effect ($F(6, 84) = 0.27$; $p = 0.95$; Figure 1a). Startle response was significantly different across dB levels ($F(6, 84) = 23.96$; $p = 0.001$). Using this protocol, PPI was disrupted in $G_s\alpha^*$ mice with a significant effect of genotype ($F(1, 14) = 9.53$; $p < 0.01$), and PPI levels were significantly different across dB levels ($F(3, 42) = 16.92$; $p < 0.001$), but no significant genotype × dB interaction was found ($F(3, 42) = 0.375$; $p = 0.77$; Figure 1b).

R(AB) transgenic mice ($n = 11$) and wild-type littermate mice ($n = 11$) were also tested with protocol 1 (Figure 2). The average R(AB) startle response was not significantly different from wild-type mice ($F(1, 20) = 1.63$; $p = 0.22$), nor was there a significant genotype × dB interaction effect ($F(6, 120) = 0.13$; $p = 0.99$; Figure 2a). Startle response was significantly different across dB levels ($F(6, 120) = 27.44$; $p < 0.001$). PPI was not disrupted in R(AB) mice compared to corresponding wild-type mice ($F(1, 20) = 0.21$; $p = 0.65$), and no significant genotype × dB interaction was found ($F(3, 60) = 0.33$; $p = 0.81$; Figure 2b). PPI levels were significantly different across dB levels ($F(3, 60) = 53.38$; $p < 0.001$).

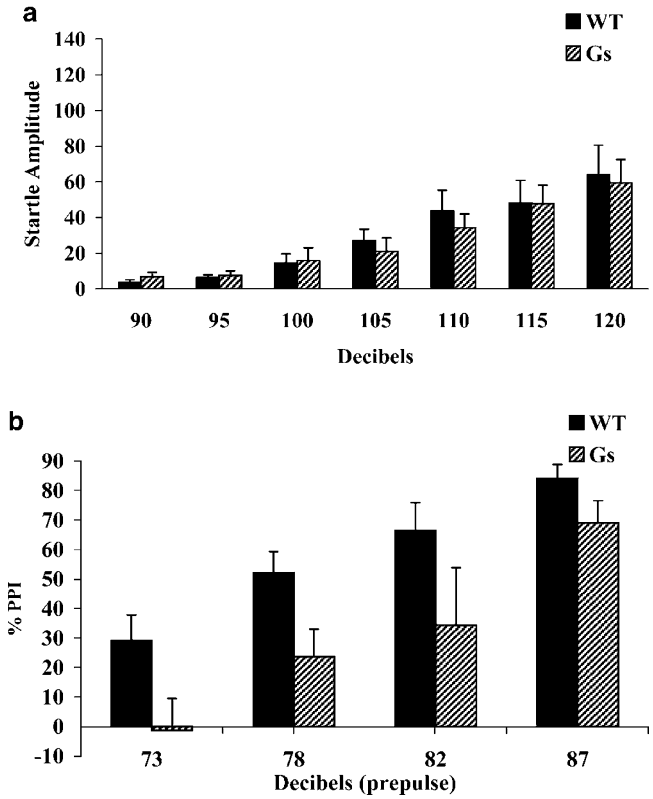

Figure 1 Prepulse inhibition and startle response of $G_s\alpha^*$ transgenic mice measured with protocol 1. (a) $G_s\alpha^*$ (Gs) and wild-type (WT) littermates did not differ in overall startle response. (b) Mice were tested at four prepulse intensities with a 120 dB startle pulse and 65 dB white noise background. Overexpression of $G_s\alpha^*$ transgene was associated with significant PPI deficits relative to WT mice.

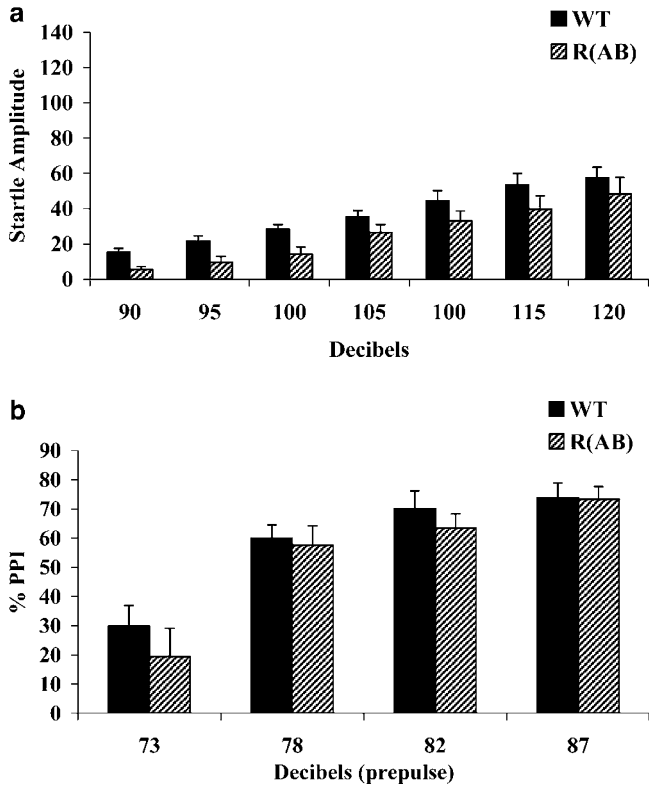

Figure 2 Prepulse inhibition and startle response of R(AB) transgenic mice measured with protocol 1. (a) Average startle response to startle stimuli ranging in decibel levels from 90–120 dB was recorded in R(AB) transgenic mice and corresponding wild-type mice (WT). The R(AB) transgenic mice did not have a deficit in acoustic startle response. (b) The R(AB) transgenic mice and corresponding wild-type mice were tested for prepulse inhibition of startle response to a 120 dB startle stimulus across a range of prepulse intensities (73–87 dB) with 65 dB background white noise. Expression of the R(AB) transgene did not alter PPI.

$G_s\alpha^*$ Transgenic Mice, but Not R(AB) Transgenic Mice, Exhibit PPI Deficits (Protocol 2)

Protocol 2 tested PPI with a 100 ms interstimulus interval between the 20 ms prepulse and the 40 ms startle stimulus. $G_s\alpha^*$ ($n = 18$) and wild-type control ($n = 18$) mice were analyzed for startle reactivity at seven different sound intensities, and tested for PPI at three different prepulse intensities, using a startle intensity of 120 dB (Figure 3). In addition, the subjects were given 'no stimulus' trials to measure the baseline movement and make the trial sequence less predictable. As found using protocol 1, the overall startle response did not differ between $G_s\alpha^*$ and wild-type mice ($F(1, 34) = 0.017$; $p = 0.90$; Figure 3a). No significant genotype × dB interaction was observed ($F(6, 204) = 0.74$; $p = 0.62$), nor were differences in baseline movement found between $G_s\alpha^*$ and wild-type mice (no stimulus trials; $t(34) = 0.42$; $p = 0.673$). Startle response was significantly different across dB levels ($F(6, 204) = 65.38$; $p < 0.001$). Prepulse inhibition was reduced in $G_s\alpha^*$ mice ($F(1, 34) = 4.74$; $p < 0.05$; Figure 3b), as observed using protocol 1. In addition, PPI levels were significantly different across dB levels ($F(2, 68) = 23.91$; $p < 0.001$), but no significant genotype × dB interaction was found ($F(2, 68) = 1.39$; $p = 0.26$).

R(AB) transgenic mice ($n = 11$) and wild-type littermate mice ($n = 11$) were also tested in protocol 2 (Figure 4). The average startle response of R(AB) transgenic mice was not significantly different from wild-type mice ($F(1, 20) = 1.19$; $p = 0.29$); no significant genotype × dB interaction effect was found ($F(6, 120) = 0.67$; $p = 0.68$), nor were there differences in baseline movement (no stimulus trials; $t(20) = 0.28$; $p = 0.78$). Startle response was significantly different across dB levels ($F(6, 120) = 20.69$; $p < 0.001$; Figure 4a). For PPI, no main effect of genotype was found ($F(1, 20) = 0.75$; $p = 0.40$), but a significant genotype × dB interaction was found ($F(2, 40) = 3.45$; $p < 0.05$; Figure 4b). The R(AB) transgenic mice had greater PPI at the 69 dB prepulse level ($p < 0.05$; Tukey corrected *post hoc* comparison). PPI levels were also significantly different across dB levels ($F(2, 40) = 41.79$; $p < 0.001$).

DISCUSSION

The endophenotypes of schizophrenia that have been studied in animals include alterations in sensorimotor gating, latent inhibition, attention, social behavior, and cognition (Baruch *et al*, 1988; Dulawa *et al*, 1997; Feldon and Weiner, 1991; Hoffman and Donovan, 1994; Kline *et al*, 1998; Martinez and Geyer, 1997; Mohn *et al*, 1999; Solomon *et al*, 1981; Swerdlow and Geyer, 1998; Weiner *et al*, 1996). Prepulse inhibition of the startle response is a form of

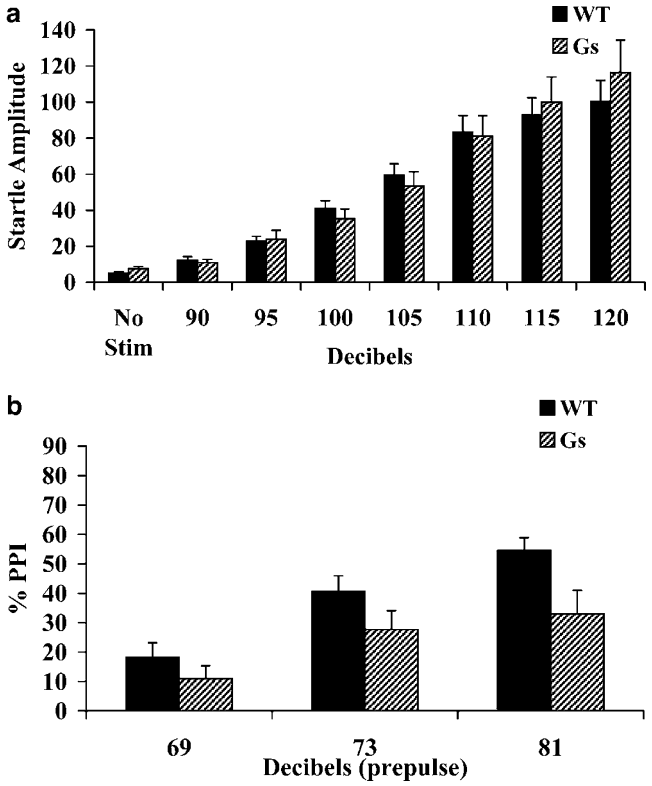

Figure 3 Prepulse inhibition and startle response of $G_s\alpha$* transgenic mice measured with protocol 2. (a) $G_s\alpha$* (Gs) and wild-type (WT) littermates did not differ in overall startle response. (b) Significant overall deficits in PPI were observed in $G_s\alpha$* mice tested at three prepulse intensities, with a 120 dB startle pulse and 65 dB white noise background.

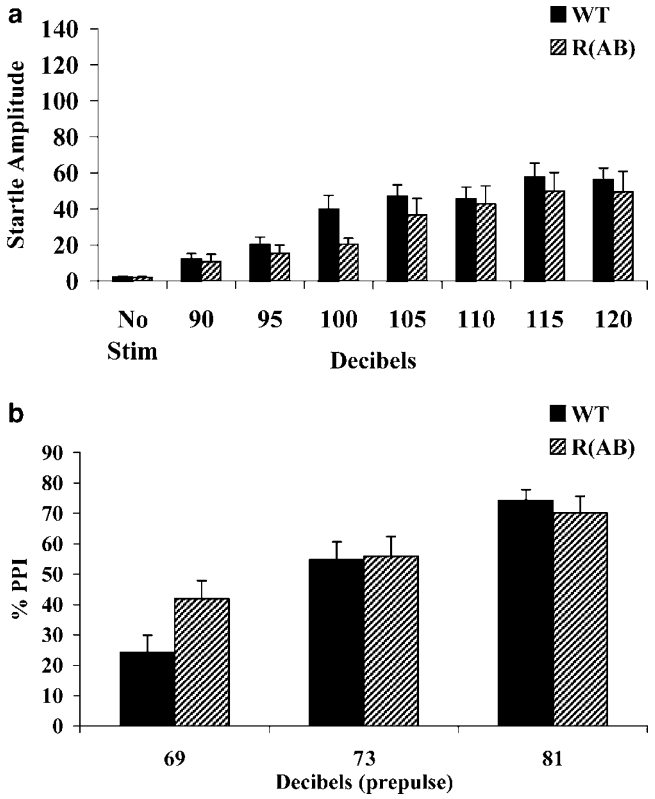

Figure 4 Prepulse inhibition and startle response of R(AB) transgenic mice measured with protocol 2. (a) The R(AB) transgenic mice did not have a deficit in acoustic startle response. (b) For PPI, an interaction between genotype and prepulse dB level existed. The R(AB) transgenic mice had a greater level of PPI compared to wild-type mice at the 69 dB prepulse level.

sensorimotor gating that has been studied intensely, because it can easily be measured in both humans and laboratory animals, and because patients with schizophrenia, Huntington's disease, obsessive compulsive disorder, Tourette's syndrome, or attention deficit disorder show reliable deficits in PPI (Geyer and Braff, 1987; Koch, 1999; Swerdlow and Geyer, 1998). The reduced PPI found in patients with schizophrenia may reflect a loss of sensorimotor gating that could serve as the basis for the sensory flooding and cognitive impairments that these patients experience. The development of animal models has been critical not only for understanding the neurological basis of psychiatric diseases, but also for the development of novel therapeutic strategies. The goal of these studies was to examine the relationship between PPI deficits and alterations of G-protein-mediated cAMP/PKA intracellular signaling, using mice that have elevated levels of G_s signaling and cAMP (Abel *et al*, 1997a; Wand *et al*, 2001), and mice that have reduced PKA activity (Abel *et al*, 1997b). Elevated levels of G_s signaling were associated with deficits in PPI. The $G_s\alpha^\star$ transgenic mice showed deficits in PPI in two different protocols that were based on protocols reported to successfully establish PPI in mice (Tarantino *et al*, 2000; Ralph *et al*, 1999). This suggests that the PPI deficits in the $G_s\alpha^\star$ transgenic mice are not dependent on the PPI protocol used. Although we have only analyzed one line of $G_s\alpha^\star$ transgenic mice in the studies reported here, our recent studies have revealed PPI deficits in other transgenic lines overexpressing $G_s\alpha$ (Kelly *et al*, 2003).

Cognitive impairments represent another important class of endophenotypes typical of schizophrenia. Specific abnormalities are present in attention, memory, and executive functions (Rund and Borg, 1999). Studies by Saykin *et al* (1991, 1994) have demonstrated a selective deficit in learning and memory compared to other cognitive areas in a sample of unmedicated patients. In these studies, verbal memory and learning deficits accounted for most of the variance between patients and controls, supporting an important role for the left-temporal hippocampal system in schizophrenia. Interestingly, although many studies of animal models have focused on sensory and sensorimotor gating, few animal studies have focused on hippocampus-dependent forms of learning and memory as potential endophenotypes of schizophrenia. It may be that an appropriate animal model of this complex disease would exhibit deficits in both learning as well as sensorimotor gating. Thus, the $G_s\alpha^\star$ mice are of particular interest because the changes in learning and memory (Abel *et al*, 1997a), sensorimotor gating, and G-protein function noted in these transgenic mice may model changes seen in schizophrenia. Experiments are underway to determine if antipsychotics ameliorate PPI deficits in the $G_s\alpha^\star$ transgenic mice (Kelly *et al*, 2003).

R(AB) transgenic mice also have disrupted hippocampus-dependent learning and synaptic plasticity (Abel *et al*, 1997b; Woo *et al*, 2002, 2000). Interestingly, the R(AB) mice did not exhibit deficits in PPI. This demonstrates that

alterations in hippocampal cAMP/PKA signaling and associated deficits in hippocampus-dependent learning do not predict deficits in PPI. These data suggest that PKA in neurons within the forebrain (cortex, hippocampus, striatum and amygdala) does not play an essential or necessary role for startle and PPI. This is in contrast to our observations of deficits in sensorimotor gating in $G_s\alpha^*$ transgenic mice in which the cAMP/PKA pathway has been constitutively activated. Combined, these experiments support the idea (discussed below) that the cAMP/PKA signaling pathway serves to modulate PPI, perhaps by acting in brain regions such as the hippocampus and or nucleus accumbens that modify the startle circuitry (Koch, 1999). However, it is important to note that these transgenes are expressed in subpopulations of neurons in specific brain regions. Thus, our results for PPI do not exclude the involvement of PKA in cell types or brain regions not expressing the transgene. Additionally, the lack of deficit in the R(AB) mice could be due to compensatory changes associated with expression of the R(AB) transgene that mask the role of PKA in PPI.

Schizophrenia-associated deficits in multiple neurotransmitter receptor subtypes may be related to common underlying mechanisms, such as changes in shared intracellular signaling pathways. Many of the neurotransmitters implicated in schizophrenia utilize G-protein coupled receptors, raising the possibility that the misregulation of G-protein signaling plays a role in the molecular changes underlying the pathophysiology of this disorder. This idea is supported by recent microarray studies that observed alterations in RGS4 in patients with schizophrenia (Chowdari *et al*, 2002; Mirnics *et al*, 2001). Our data demonstrate that $G_s\alpha^*$ transgenic mice, which overexpress $G_s\alpha^*$, exhibit a deficit in PPI without changes in the startle response itself. These results strongly imply that overactivation of G_s signaling can cause sensorimotor gating abnormalities.

The mechanism by which overexpression of $G_s\alpha^*$ disrupts PPI is not clear, but it could relate to the modulation of neurotransmitter systems such as the dopaminergic system. Both clinical and animal studies of PPI have defined a role for dopamine in this form of startle plasticity. Indirect dopamine agonists, such as amphetamine, and direct dopamine agonists, such as apomorphine, disrupt PPI in rats (Mansbach *et al*, 1988) and mice (Ralph *et al*, 1999). Importantly, these disruptions in PPI are reversed by typical and atypical antipsychotics, and the ability of antipsychotics to restore PPI strongly correlates with their clinical potency (Swerdlow *et al*, 1994). Numerous studies have demonstrated dopamine agonist-induced disruption of PPI, and this effect appears to be dependent on the D_2 receptor (Ralph *et al*, 1999). However, increasing evidence demonstrates a role of the D_1 receptor in PPI. In mice, the D_1 agonist SKF82958 significantly reduced PPI and the D_1 antagonist SCH23390 blocked the mixed D_1/D_2 agonist apomorphine disruption of PPI (Ralph-Williams *et al*, 2003). Thus, the roles of D_1 and D_2 receptor subtypes in disruption of PPI still need to be resolved. In humans, the D_2 agonist pergolide failed to disrupt PPI (Braff *et al*, 2001; Swerdlow *et al*, 2002). The effects of direct D_1 agonists on PPI in humans have not been reported. Because the D_1 receptor is coupled to G_s, overexpression of $G_s\alpha^*$ may produce effects on PPI similar to D_1 agonists. In the $G_s\alpha^*$ transgenic mice, overexpression of the $G_s\alpha$ transgene is limited to forebrain areas that include the hippocampus, nucleus accumbens, and prefrontal cortex, suggesting that the modulation of PPI is due to altered cAMP signaling in one of these brain regions.

Overexpression of $G_s\alpha^*$ could increase neural activity that drives dopamine release in brain areas involved in PPI. The nucleus accumbens is a subcortical integrative center that connects forebrain structures such as the hippocampus, prefrontal cortex, and amygdala to circuits that mediate PPI. The nucleus accumbens receives a dense dopaminergic projection from the ventral tegmental area, and excessive dopamine receptor stimulation within the nucleus accumbens reduces PPI (reviewed in Koch, 1999; Swerdlow and Geyer, 1998). Dopamine release in the nucleus accumbens is controlled in part by glutamatergic afferents from the limbic system. Glutamatergic neurons from the hippocampus and medial prefrontal cortex project to the nucleus accumbens. Hippocampal fibers enhance dopamine release from ventral neurons in the shell region of the nucleus accumbens via a direct presynaptic mechanism mediated by glutamate, and PPI is disrupted after stimulation of the ventral hippocampus. The medial prefrontal cortex projects to the core region of the nucleus accumbens, where these projections enhance dopamine release. Since a disturbance in the interaction between glutamate and dopamine is thought to play a role in the pathophysiology of schizophrenia, the nucleus accumbens is a prominent structure in models of sensory gating deficits in patients.

Because startle was not altered in the transgenic mice, this suggests that the disruption of PPI in $G_s\alpha^*$ transgenic mice was not likely to be due to changes in the brainstem, but more likely to be due to changes in forebrain areas (see Koch, 1999). The hippocampal–accumbal projection appears to be a crucial regulator of dopamine release within the accumbens. Previous electrophysiological experiments found enhanced basal synaptic transmission within the hippocampus of the $G_s\alpha^*$ transgenic mice (Abel *et al*, 1997a). If synaptic transmission in the hippocampal–accumbal pathway is also enhanced, then transgenic mice would be expected to have elevated levels of dopamine within the accumbens, thus resulting in a deficit in PPI. Although the dopamine system, in particular, is a central player in the modulation of PPI, other neurotransmitter systems, including serotonin, noradrenaline, and glutamate, also play a role in PPI. Thus, it is possible that disrupted PPI in the $G_s\alpha^*$ transgenic mice is a result of alterations to one or more neurotransmitter systems. Future experiments will be directed at identifying the neurotransmitter systems and brain regions involved.

The $G_s\alpha^*$ transgenic mice may provide an interesting animal model of schizophrenia. These mice have altered G-protein signaling that models the imbalance of G-protein signaling observed in schizophrenia (Nishino *et al*, 1993). In addition, the $G_s\alpha^*$ transgenic mice have deficits in both PPI and hippocampus-dependent spatial learning, similar to deficits associated with schizophrenia (Geyer and Braff, 1987; Rund and Borg, 1999; Swerdlow and Geyer, 1998). The study of altered G-protein signaling as a potential underlying cause of schizophrenia has potentially important clinical and pharmacological ramifications, and it will be

interesting to examine the effects of antipsychotics on the PPI deficits in $G_s\alpha^*$ transgenic mice.

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