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MICE EXPRESSING CONSTITUTIVELY ACTIVE $G_s\alpha$ EXHIBIT STIMULUS ENCODING DEFICITS SIMILAR TO THOSE OBSERVED IN SCHIZOPHRENIA PATIENTS

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Abstract

People with schizophrenia display sensory encoding deficits across a broad range of electrophysiological and behavioral measures, suggesting fundamental impairments in the ability to transduce the external environment into coherent neural representations. This inability to create basic components of complex stimuli interferes with a high fidelity representation of the world and likely contributes to cognitive deficits. The current study evaluates the effects of constitutive forebrain activation of the $G_s\alpha$ G-protein subunit on auditory threshold and gain using acoustic brainstem responses and cortically generated N40 event-related potentials to assess the role of cyclic AMP signaling in sensory encoding. Additionally, we examine the ability of pharmacological treatments that mimic (amphetamine) or ameliorate (haloperidol) positive symptoms of schizophrenia to test the hypothesis that the encoding deficits observed in $G_s\alpha$ transgenic mice can be normalized with treatment. We find that $G_s\alpha$ transgenic mice have decreased amplitude of cortically generated N40 but normal acoustic brainstem response amplitude, consistent with forebrain transgene expression and a schizophrenia endophenotype. Transgenic mice also display decreased stimulus intensity response (gain) in both acoustic brainstem response and N40, indicating corticofugal influence on regions that lack transgene expression. N40 deficits in transgenic animals were ameliorated with low dose haloperidol and reversed with higher dose, suggesting dopamine D_2 receptor-linked G_i activity contributes to the impairment. Consistent with this hypothesis, we recreated the $G_s\alpha$ transgenic deficit in wild type animals using the indirect dopamine agonist amphetamine. This transgenic model of sensory encoding deficits provides a foundation for identifying biochemical contributions to sensory processing impairments associated with schizophrenia.

Keywords

$G_s\alpha$; N40; schizophrenia; cAMP; haloperidol; event-related potentials

People with schizophrenia have difficulty interpreting events and stimuli in their environment. Such difficulties are dramatically manifest as delusions of reference and sensory illusions but may also result in confusion and inability to piece together signals required to interact in society. This integrated process of detecting and interpreting the constellation of multimodal stimuli is too complex to understand as a singular phenomenon or study with interpretable measures in the human or animal laboratory. However, evidence

from multiple investigators using complimentary approaches indicates that the inability to interpret complex stimuli may begin with abnormalities in early detection and encoding of stimulus characteristics. Disruption of early neural processes likely degrades the building blocks of interpretation and is consistent with abnormalities in cognitive domains that require qualitative assessment of stimulus parameters on which to base decisions. If a signal is misallocated with respect to its relevance, its encoding in memory will be altered and decisions based on distorted information.

Event-related potentials (ERPs) allow for evaluation of sensory processing in which the latency of potentials is determined by generators of neural activity. In humans, early ERPs (1–8 ms) represent activation of brainstem structures such as the cochlear nucleus; mid-latency ERPs (8–40 ms) indicate forebrain activity including thalamus, hippocampus and primary auditory cortex and long-latency ERPs (50–300 ms) represent higher processing, involving primary and association cortices (Picton et al., 1974). Patients with schizophrenia display abnormalities in auditory processing including reductions in amplitude of long-latency cortical ERP components. One report indicates that patients with schizophrenia demonstrate impaired cortical ERPs with no differences in brainstem-generated potentials (Pfefferbaum et al., 1980; Boutros et al., 2004). Additional ERP components including mismatch negativity have been proposed as predictive markers of cognitive function in patients with schizophrenia (Boutros et al., 2004; Light and Braff, 2005a,b).

Early, middle and long-latency ERPs recorded from mice resemble corresponding human components in polarity and response properties (Connolly et al., 2003; Maxwell et al., 2004a; Umbricht et al., 2004; Siegel et al., 2005). As such, ERPs in mice provide useful models for genetic and mechanistic understanding of brain abnormalities and treatment development in schizophrenia. Specifically, rodents share many similarities with humans for specific portions of the ERP, including the mouse P1, N1, P2 and P3. These components are also named the P20, N40, P80 and P120 for latency and share stimulus and pharmacologic response properties with the human P50, N100, P200 and P300 respectively (Siegel et al., 2003, 2005; Connolly et al., 2004; Maxwell et al., 2004b; Umbricht et al., 2005).

Previous reports indicate that auditory ERPs are modulated by compounds that influence the cyclic AMP (cAMP) signaling cascade. For example, antipsychotic compounds, which regulate cAMP production through dopamine D₂ receptor blockade, and phosphodiesterase inhibitors, which prevent cAMP hydrolysis, increase auditory ERP amplitude (Maxwell et al., 2004a,b). Conversely, the indirect dopamine agonist amphetamine decreases ERP amplitude (Maxwell et al., 2004a). Additionally, G-protein-coupled receptors including G_s and G_i that influence intracellular cAMP concentrations modulate sensory-motor gating in rodents (Culm et al., 2003; Gould et al., 2004; Pineda et al., 2004). Therefore, the G-protein-coupled cAMP signaling cascade is a target for evaluating auditory ERPs to help understand sensory processing deficits similar to those in schizophrenia. The current study utilizes a mouse model of schizophrenia that limits constitutively active G_sα transgene expression to postnatal forebrain structures through use of a CaMKIIα promoter, providing a degree of anatomic localization for observed abnormalities.

EXPERIMENTAL PROCEDURES

Animals/surgery

G_sα transgenic mice were bred and group-housed at the University of Pennsylvania in a hemizygous state on a C57BL/6 background (N12–N14). As previously described, G_sα* transgenic mice express an isoform of the G-protein subunit G_sα that is constitutively active due to a point mutation (Q227L) that prevents hydrolysis of bound GTP (Wand et al., 2001; Gould et al., 2004). Expression of the transgene is driven by the CaMKIIα-promoter, which

restricts expression to postnatal forebrain neurons. Specifically, *in situ* hybridization studies indicate that the transgene is expressed in striatum, hippocampus and cortex but not cerebellum, thalamus or brainstem (Wand et al., 2001). Animals were genotyped by Southern blot using a transgene-specific probe as described (Abel et al., 1997). All protocols were conducted in accordance with University Laboratory Animal Resources guidelines and were approved by the National Institutes of Health. Animals were individually housed following surgery in a light and temperature-controlled Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal facility. Food and water were available *ad libitum*. All efforts were made to minimize animal numbers and suffering.

Thirteen wild type (six male, seven female) and 11 transgenic G_{α}^* (five male, six female) mice aged 10–13 weeks were implanted with unipolar electrodes for non-anesthetized recordings of whole brain EEG activity two weeks later (Maxwell et al., 2004a). We have previously shown that this recording method closely resembles human scalp EEG recordings (Siegel et al., 2003). Animals were anesthetized with isoflurane. Recording electrodes were placed in the CA3 hippocampal region, (1.4 mm posterior, 2.65 mm lateral and 2.75 mm deep relative to bregma) and referenced to the ipsilateral frontal sinus. The electrode pedestal was secured to the skull using dental cement and superglue. Electrode placement was verified to be in the target region using the Perl's iron reaction (LaBossiere and Glickstein, 1976).

Experimental design

This study consisted of three experiments that had a within animal design. Experiment 1: Genotype differences were determined by evaluating the average across two saline trials administered into the i.p. cavity and conducted one week apart. Experiment 2: Vehicle, 0.1 mg/kg and 1 mg/kg haloperidol (Sigma) were administered i.p. to examine the ability of a known antipsychotic medication to reverse baseline differences in auditory evoked potentials. Experiment 3: Vehicle, 0.1 mg/kg, 0.5 mg/kg and 1 mg/kg *d*-amphetamine (Sigma) were administered i.p. to evaluate the hypothesis that the transgene would recreate both the effects of amphetamine seen in wild type mice as well as those seen in untreated patients with schizophrenia. Experiments 2 and 3 were performed in a counterbalanced fashion in which half of the animals received drug and half received vehicle on each test day. Age- and gender-matched wild type littermates were run in parallel to G_{α}^* transgenic mice. Additionally, animals were given at least 48 h between drug exposures.

Auditory evoked potentials

The generation of a positive deflection at 3 ms (P3) and a negative deflection at 5 ms (N5) poststimulus is thought to represent activation of hindbrain structures including the auditory nerve and brainstem based on the hypothesis that latency of the evoked potential reflects relative generation site (Henry, 1979; Connolly et al., 2003). Similarly, a negative deflection occurring at 40 ms (N40) poststimulus indicates activation of forebrain structures including hippocampus, thalamus and auditory cortex (Maxwell et al., 2004b; Mamiya et al., 2005). Evaluations of gene effects on the P3N5 complex and the N40 allow for regional assessments of brainstem responses and higher cortical processing of auditory stimuli.

Brainstem response

Auditory brainstem responses were evaluated prior to injection on each testing session at six stimulus intensities to determine baseline differences in brainstem-evoked potentials. Consistent with previous publications, the protocol consists of 1000 white noise stimuli (3 ms duration) presented at intensities of 0, 58, 66, 74, 82 and 90 dB (Connolly et al., 2003). These stimulus intensities were chosen to provide a thorough assessment of auditory threshold. Raw EEG data were sampled at 6250 Hz using Micro 1401 hardware and spike 5

software (Cambridge Electronic Design, Cambridge, UK). Waveforms were filtered between 1 and 300 Hz, baseline corrected at stimulus onset, and average waveforms were made from 2 ms prior to stimulus presentation through 9 ms after stimulus onset. As previously published, the amplitude of the P3 (2–4 ms window) to N5 (3–6 ms window) difference waveform was analyzed using a repeated measure ANOVA to examine main effects of genotype and interactions between genotype and intensity (Connolly et al., 2003).

Cortical processing

Fifty white noise stimuli (10 ms duration) were presented with an 8 s inter-stimulus interval at intensities of 75, 85 and 95 dB compared with a background of 70 dB. These stimulus intensities were chosen to provide a wide range of intensities within the time limitations during which the pharmacological treatments were effective. As such, auditory brainstem responses were not evaluated following drug treatment in these animals. Raw EEG data were sampled at 1667 Hz. Waveforms were baseline corrected at stimulus onset and individual trials rejected for movement artifact based on twice the root mean squared amplitude per mouse. Average waveforms were created from 50 ms pre-stimulus to 200 ms post stimulus (Fig. 1).

The amplitude of the N40 (25–60 ms w and drug (Statistica; Statsoft, Tulsa, OK, USA). Significant interactions were followed with Fisher LSD post hoc analyses. Initial analyses of N40 amplitude included gender as a variable. No significant effects of gender or interactions between gender and other variables were found on N40 amplitude at baseline or with either drug condition. Therefore, the gender variable was removed from this analysis in order focus on the effects of genotype.

RESULTS

We previously reported that constitutive activation of $G_s\alpha$ causes impairments in pre-pulse inhibition (PPI) and proposed this mutation as a model of schizophrenia-like sensory-motor gating deficits (Gould et al., 2004). The current study demonstrates the effects of the forebrain-specific $G_s\alpha$ transgenic manipulation on auditory ERPs to determine regional contributions to specific phases of sensory encoding deficits relevant to schizophrenia. Additionally, we test the hypothesis that pharmacological treatments that ameliorate positive symptoms of schizophrenia (hallucinations and delusions) will normalize sensory encoding deficits caused by the $G_s\alpha$ mutation. These studies provide a novel transgenic mouse model of sensory encoding deficits associated with schizophrenia and may facilitate understanding of neural and biochemical mechanisms that contribute to its pathophysiology.

$G_s\alpha$ transgenic mice display reduced acoustic brainstem response (ABR) intensity response but not threshold

The P3N5 complex was analyzed to determine baseline transgene effects at the brainstem prior to acute treatments (Table 1). The amplitude of the P3N5 complex increases proportionally with stimulus intensity as evidenced by a main effect of stimulus intensity (Fig. 2). No main effect of genotype was found on this component. Although there were no significant differences between genotypes at any single intensity, a genotype by intensity interaction suggests that there are differences in the slope of the stimulus intensity function between genotypes (Table 1: $\text{gene} \times \text{intensity}$, $P=0.021$; Fig. 2A). Fisher LSD post hoc analyses indicate that wild type mice show significant increases in the P3N5 response with smaller increases in stimulus intensity (58 dB vs. 66 dB: $P=0.063$; 58 dB vs. 74 dB: $P<0.001$; 58 dB vs. 82 dB: $P<0.001$; 58 dB vs. 90 dB: $P<0.001$) than do transgenic mice (58 dB vs. 66 dB: $P=0.518$; 58 dB vs. 74 dB: $P=0.164$; 58 dB vs. 82 dB: $P=0.013$; 58 dB vs. 90 dB: $P<0.001$). ABR data were also analyzed by calculating the slope of the ABR intensity

function for each mouse. We then used a two-tailed *t*-test to compare wild type to transgenic mice for this alternate method of assessment. The average slope was 0.064 for wild type and 0.034 for transgenic with a *P*-value of 0.026. These data indicate that there are no significant differences in auditory threshold between wild type and transgenic animals but these animals differ in the stimulus intensity response (gain). Such data suggest that there are no abnormalities in the mechanisms of detecting stimuli at the level of the cranial nerves and brainstem in transgenic animals, but that the ability to encode stimulus intensity by recruiting more neurons to fire in synchrony is impaired.

G_sα transgene expression impairs cortical N40 processing

We also measured the amplitude of the mouse N40 ERP, which shares homology with the human N100 primary cortical response (Connolly et al., 2003, 2004; Siegel et al., 2003, 2005; Maxwell et al., 2004a,b). Transgenic animals had smaller N40 amplitude than wild type mice as indicated by a main effect of genotype. This component also increased in amplitude in proportion to increasing stimulus intensity. Additionally, a genotype by intensity interaction demonstrated that transgenic mice had reduced amplitude at 85 and 95 dB, suggesting an impaired stimulus intensity response (Fig. 2B).

G_sα transgenic deficit is reversed with haloperidol

The hypothesis that antipsychotic treatment would reverse the transgenic deficit, as shown in pharmacological models of schizophrenia, was tested by administration of haloperidol (Maxwell et al., 2004a,b; Siegel et al., 2005). Both doses of haloperidol increased the N40 amplitude in transgenic mice (Table 1: genotype×drug *P*=0.003). The 0.1 mg/kg dose of haloperidol increased the N40 amplitude in G_sα transgenic mice to match that of wild type littermates. The 1.0 mg/kg dose of haloperidol enhanced N40 amplitude in G_sα transgenic mice above that of wild type littermates (transgenic vs. wild type: vehicle *P*=0.033; 0.1 mg/kg *P*=0.760; 1.0 mg/kg *P*=0.038, Fig. 3A), suggesting that the higher dose unmasked an underlying augmentation from unopposed G_sα activity.

G_sα transgenic deficits are recreated in wild type mice using amphetamine

Amphetamine was administered to assess the hypothesis that increased dopamine activity at both D1 and D2-like DA receptors would recreate the G_sα transgenic deficit in wild type animals. A genotype by stimulus intensity interaction revealed the baseline stimulus intensity deficit in transgenic relative to wild type animals (Fig. 2D). Wild type mice displayed a dose-dependent decrease in amplitude with amphetamine whereas the transgenic animals were unchanged (Fig. 3B). A genotype by amphetamine by intensity interaction revealed that all doses of amphetamine prevented wild type animals from displaying a normal stimulus intensity response which resembled the transgenic impairment (Table 2).

DISCUSSION

Overall, G_sα transgenic mice displayed decreased amplitude of cortically-generated N40 with normal ABR amplitude, consistent with forebrain transgene expression and the schizophrenia endophenotype. G_sα transgenic mice also displayed decreased stimulus intensity response (gain) for both ABR and N40 amplitude, indicating corticofugal influence on brainstem regions that lack transgene expression. N40 deficits in G_sα transgenic animals were ameliorated with haloperidol 0.1 mg/kg and reversed with the 1.0 dose, suggesting activity of dopamine D₂ receptor-linked G_i G-protein contributes to the observed impairment. Consistent with this hypothesis, the indirect dopamine agonist amphetamine recreated the G_sα transgenic deficit in wild type animals.

G_sα transgenic deficit in forebrain processing consistent with transgene expression

The patterns of normal ABR thresholds and impaired N1 (mouse N40, human N100) cortical ERP amplitude are consistent with findings in patients with schizophrenia (Pfefferbaum et al., 1980). Results using ABRs indicate that transgenic and wild type animals do not differ in amplitude for the lowest intensity stimuli at the level of cochlea or brainstem, suggesting normal auditory threshold. However, the decreased N40 amplitude in transgenic animals provides evidence for impairments in auditory encoding of qualitative stimulus features such as intensity in cortical regions, indicating that this mutation is manifest in higher order processing and/or the neuronal gain function. This hypothesis is consistent with the pattern of targeted transgene expression in postnatal forebrain neurons (Wand et al., 2001; Gould et al., 2004).

Genotype influences stimulus intensity response

Interestingly, the slope of the stimulus intensity response is reduced in both the brainstem and cortical measures in G_sα transgenic mice. Specifically, the degree to which the stimulus intensity response increases differs with genotype such that the difference between softer and louder tones is less significant in transgenic mice. Because the transgene is not expressed in brainstem, this reduction in ABR gain function may reflect descending corticofugal forebrain influences on hindbrain processing. This finding indicates that although the transgene has limited expression, the G_sα mutation may alter multiple phases of auditory processing and impact early, middle and late latency-evoked potentials. Based on these data, we propose that forebrain structures mediate normal stimulus intensity responses throughout the auditory pathway. This finding has implications for auditory processing deficits related to schizophrenia. Because most studies in patients with schizophrenia use a single stimulus intensity, these data provide evidence for the need to examine the stimulus intensity responses in patients to more thoroughly characterize the auditory processing impairments (Light and Braff, 1999; Boutros et al., 2004).

Antagonism of G_i-coupled D₂ DA receptors normalizes G_sα transgenic deficit

The G_i-coupled dopamine D₂-receptor antagonist haloperidol normalized the transgenic deficit at the low dose (0.1 mg/kg) and resulted in augmentation of the N40 amplitude in transgenic animals beyond that of wild type mice at the higher dose (1 mg/kg). Importantly, this same dose of haloperidol also rescued the PPI deficits exhibited by these mice (Kelly et al., in press). We propose that this increased amplitude in transgenic mice following G_i-coupled D₂-blockade likely results from the unopposed actions of constitutively active G_s on auditory processing. This suggests that the baseline ERP and PPI deficits in transgenic mice may result from compensatory changes that mimic or produce increased D₂-mediated dopamine function. Thus, our model demonstrates that primary alterations in D_{1,5} receptor-mediated signal transduction can produce schizophrenia-like deficits in neural function that are reversed with antipsychotic medication.

Dopamine facilitation recreates G_sα transgenic deficit in wild type mice

The indirect dopamine agonist amphetamine produces schizophrenia-like deficits in ERPs in both humans and animals (Adler et al., 1986; Light et al., 1999). Although amphetamine decreased the N40 amplitude in wild type mice, it did not produce further decrements in G_sα transgenic littermates. This result suggests that transgenic mice may have increased dopaminergic tone at baseline, obscuring the ability to produce further increases. Again, these data suggest that an initial increase in G_sα function produced G_i-coupled, D₂-mediated deficits similar to those seen in schizophrenia.

CONCLUSION

In summary, our data provide a transgenic mouse model of schizophrenia-like auditory processing deficits that are ameliorated with haloperidol. Additionally, we have recreated this transgenic deficit using amphetamine in wild type littermates, suggesting a role for increased catecholamine release or function in the abnormal auditory processing profile. This model can potentially be used to screen pharmacological compounds that may improve fundamental sensory processing abnormalities which underlie higher-order cognitive impairments associated with schizophrenia. This study provides support for the involvement of abnormal G-protein-mediated intracellular cAMP signal transduction in sensory processing of schizophrenia.

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Abbreviations

ABR	acoustic brainstem response
cAMP	cyclic AMP
ERP	event-related potential
PPI	pre-pulse inhibition

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Fig. 1.

Grand average waveforms for $G_s\alpha$ transgenic and wild type mice in each pharmacological condition. Baseline ERP waveforms for wild type (black) and $G_s\alpha$ (gray) mice are shown in panel A. Note that transgenic mice have significantly smaller N40 amplitude than wild type littermates under basal conditions. Panel B demonstrates that haloperidol 0.1 mg/kg normalizes N40 amplitude in $G_s\alpha$ mice relative to Wt mice. As shown in panel C, haloperidol 1.0 mg/kg causes a reversal of the baseline difference in N40 amplitude such that $G_s\alpha$ transgenic animals have significantly larger N40 than wild type littermates under more complete D2 antagonism. Amphetamine causes a dose dependent decrease in N40 amplitude among wild type mice as shown in panels D (0.1 mg/kg), E (0.5 mg/kg) and F (1.0 mg/kg). The N40 is marked with an arrow in panel A.

Fig. 2.

$G_{s\alpha}$ mice have normal auditory threshold with reduced ABR and N40 gain. ABR (mean +SEM) measured as the P3–N5 amplitude in wild type and transgenic mice is shown at 58–90 dB in panel A. Note that there is no overall difference in ABR amplitude between transgenic and wild type mice, indicating there is no deficit for auditory threshold or at any individual intensity. Comparison of ABR amplitude between wild type (black) vs. transgenic (gray) mice at each intensity using Fisher LSD post hoc analyses ($M_s=0.56$, $df=84$) are 58 dB ($P=0.456$), 66 dB ($P=0.846$), 74 dB ($P=0.733$), 82 dB ($P=0.441$), 90 dB ($P=0.281$). However, there are differences in the amount of increased in ABR amplitude with increasing stimulus intensity (gain) within each genotype. Panel B shows baseline reduction in N40 amplitude and intensity function (gain) in $G_{s\alpha}$ transgenic mice relative to wild type littermates. Note that transgenic mice have significantly lower amplitude of N40 response at both 85 and 95 dB, but not at 75 dB ($M_s=1732$, $df=61$), 75 dB ($P=0.849$), 85 dB ($P=0.006$), and 95 dB ($P<0.001$). Panel C demonstrates that haloperidol (across doses) reverses the $G_{s\alpha}$ transgenic deficit for N40 amplitude. Panel D demonstrates that amphetamine (across doses) causes a reduction in the overall amplitude and intensity function for N40 amplitude (gain) for wild type but not transgenic mice ($M_s=969$, $df=37$) 75 dB ($P=0.761$), 85 dB ($P=0.105$), and 95 dB ($P=0.033$). Note the similarity between panels B and D for overall pattern of N40 intensity function. Taken together, these data suggest that the $G_{s\alpha}$ transgene and increased DA activity result in a schizophrenia-like pattern of reduced N1 amplitude. Wild type mice are shown in black and $G_{s\alpha}$ transgenic mice in gray in all panels. * Indicates $P<0.05$ for wild type vs. transgenic mice.

Fig. 3.

$G_s\alpha$ transgenic endophenotype is reversed by haloperidol and mimicked by amphetamine: Panel A displays the interaction between haloperidol and genotype for N40 amplitude. Note that transgenic mice have significantly reduced N40 amplitude at baseline, with no significant difference between genotypes after 0.1 mg/kg haloperidol, and the 1.0 mg/kg dose of haloperidol resulting in reversal of baseline deficits, with $G_s\alpha$ transgenic mice displaying significantly larger N40 amplitude than wild type littermates ($MS=2533$, $df=65$). These data suggest that full D_2 type DA receptor antagonism unmasks the selective effect of the $G_s\alpha$ transgene on auditory processing. Panel B shows the percent change relative to vehicle for both doses of haloperidol. Panel C displays the interaction between amphetamine and genotype for N40 amplitude. $G_s\alpha$ transgenic mice have significantly reduced amplitude at baseline, as found in both experiments 1 and 2. However, the difference between genotypes is eliminated at all doses of amphetamine due to a dose dependent reduction in N40 amplitude among wild type but not transgenic mice ($MS=1568$, $df=71$). * Indicates $P<0.05$ for wild type (black) vs. transgenic (gray). Panel D shows the percent change relative to vehicle for each dose of amphetamine.

Table 1

Statistical values for each experiment

Variable	Auditory threshold (ABR)	Experiment 1: N40 baseline	Experiment 2: N40 haloperidol	Experiment 3: N40 amphetamine
Genotype (1)	$F(1, 22) < 0.02, P = 0.898$	$F(1, 22) = 23, P < 0.001$	$F(1, 22) = 0.02, P = 0.892$	$F(1, 22) = 6.1, P = 0.022$
Intensity (2)	$F(5, 110) = 89, P < 0.001$	$F(2, 44) = 22, P < 0.001$	$F(2, 44) = 27, P < 0.001$	$F(2, 44) = 45, P < 0.001$
Drug exposure (3)	—	—	$F(2, 44) = 11, P < 0.001$	$F(3, 66) = 11, P < 0.001$
1×2	$F(5, 110) = 2.8, P = 0.021$	$F(2, 44) = 11, P < 0.001$	$F(2, 44) = 3.0, P = 0.061$	$F(2, 44) = 6.1, P = 0.004$
1×3	—	—	$F(2, 44) = 6.5, P = 0.003$	$F(3, 66) = 3.8, P = 0.015$
2×3	—	—	$F(4, 88) = 1.4, P = 0.241$	$F(6, 132) = 4.4, P < 0.001$
1×2×3	—	—	$F(4, 88) = 0.7, P = 0.617$	$F(6, 132) = 3.9, P = 0.001$

Variable 1 indicates genotype, variable 2 indicates intensity and variable 3 indicates drug condition (haloperidol or amphetamine) for experiments 2 and 3. Note, there are only two variables (genotype and intensity) in the baseline and threshold experiments. Significant main effects and interactions are highlighted in bold.

Table 2

Amphetamine by gene by intensity interaction for N40 amplitude

Amphetamine (mg/kg)	Stimulus intensity (dB)	Wild type	Transgenic	<i>P</i> -value
0	75	21±7	22±8	0.973
	85	89±17	29±19	0.133
	95	181±24	54±26	0.003
0.1	75	48±12	26±13	0.568
	85	77±18	47±19	0.436
	95	107±21	80±23	0.484
0.5	75	21±6	15±7	0.881
	85	47±11	12±12	0.360
	95	64±13	34±14	0.440
	75	13±7	17±8	0.935
1	85	15±7	12±8	0.926
	95	33±10	43±11	0.800

Note that amphetamine dose dependently decreases the normal intensity function (gain) for N40 amplitude in wild type but not transgenic animals. Fisher LSD post hoc analyses ($MS=2531$, $df=77$). *P*-values are listed for wild type vs. transgenic mice.