Genetically Determined Differences in Learning from Errors

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pest pink bollworm (*Pectinophora gossypiella*). Larvae from AZP-R, but not APHIS-S, survived on Bt cotton producing Cry1Ac (*8*, *9*). This resistance to Cry1Ac in AZP-R is linked with deletion mutations in the cadherin receptor gene (*10*). Cry1AbMod and Cry1AcMod reduced or overcame resistance in the AZP-R strain (Fig. 3 and Table 1). On the basis of the concentration that killed 50% (LC_{50}) for AZP-R relative to APHIS-S, AZP-R was >910-fold resistant to Cry1Ab and >3700-fold resistant to Cry1Ac (Table 1). In contrast, AZP-R was only 2.8-fold resistant to Cry1AbMod and was not resistant to Cry1AcMod (Table 1). Against resistant larvae, the LC_{50} was more than 100 times higher for Cry1Ac than for Cry1AbMod or Cry1AcMod (Table 1). Conversely, against susceptible larvae, the native toxins were more potent than the modified toxins. This implies that, relative to native toxins against susceptible larvae, modified toxins had lower stability in the midgut, reduced oligomer-forming ability (Fig. 1), or reduced ability of oligomers to ultimately cause mortality.

The results suggest that in two species of Lepidoptera, cadherin receptor protein in the larval midgut mediates the toxicity of Cry1A toxins by facilitating removal of helix α-1, which promotes toxin oligomerization. The modified toxins Cry1AbMod and Cry1AcMod lacking helix α-1 formed oligomers in vitro without cadherin, whereas native Cry1Ab and Cry1Ac did not. The modified toxins killed insects with greatly reduced susceptibility to native Cry1A toxins caused by RNAi silencing of the cadherin gene or by mutations in the cadherin gene. These results support the pore-formation model (*15*) and not the signaling model, which does not include removal of helix α-1 or toxin oligomerization (*16*).

If the results seen here with the pink bollworm extend to other lepidopterans, Cry1AbMod and Cry1AcMod could be broadly useful for countering or delaying pest resistance to Cry1A toxins. However, we do not know whether Cry1AMod toxins kill insects with mechanisms of resistance unrelated to cadherin, such as the disruption of other receptors or decreased protease activation (*22*). Many Bt toxins have structural topology similar to Cry1A, form oligomers, and induce pores (*17*, *23*–*28*), suggesting that they share a similar mode of action. It remains to be determined whether, parallel to results with Cry1A toxins, other Cry toxins lacking helix α-1 can kill resistant insects that have altered receptors. In addition, insects can probably evolve resistance to modified Bt toxins lacking helix α-1. Nonetheless, along with native Bt toxins such as Cry2 and Vip3 (*29*) that have not been used as extensively as Cry1A toxins, the modified toxins broaden the options for pest control.

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Genetically Determined Differences in Learning from Errors

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The role of dopamine in monitoring negative action outcomes and feedback-based learning was tested in a neuroimaging study in humans grouped according to the dopamine D2 receptor gene polymorphism DRD2-TAQ-IA. In a probabilistic learning task, A1-allele carriers with reduced dopamine D2 receptor densities learned to avoid actions with negative consequences less efficiently. Their posterior medial frontal cortex (pMFC), involved in feedback monitoring, responded less to negative feedback than others' did. Dynamically changing interactions between pMFC and hippocampus found to underlie feedback-based learning were reduced in A1-allele carriers. This demonstrates that learning from errors requires dopaminergic signaling. Dopamine D2 receptor reduction seems to decrease sensitivity to negative action consequences, which may explain an increased risk of developing addictive behaviors in A1-allele carriers.

"You learn from your mistakes," people say. We usually learn from both positive and negative action outcomes, which induce reinforcement of successful and avoidance of erroneous behavior, respectively (*1*). The relative amount of learning from successes and errors varies across individuals as a result of disease or pharmacological intervention (*2*). Can even our genetic makeup influence the way we learn from errors? An important factor in the use of negative and positive feedback for learning seems to be the neurotransmitter dopamine (*3*–*5*). A human genetic polymorphism (DRD2-TAQ-IA) is known to modulate dopamine D2 receptor density. The A1 allele is associated with a reduction in D2 receptor density by up to 30% (*6*–*8*). This reduction has been linked to multiple addictive and compulsive behaviors (*9*, *10*), which suggests some insensitivity to negative consequences of self-destructive behavior. This might be linked to

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a general deficit in learning from errors. Here, we report patterns of brain activity underlying a reduced ability to use negative feedback for avoidance learning in carriers of the A1 allele. Our findings suggest a genetically driven change in the dynamic interaction of performance monitoring and long-term memory formation. When action outcomes call for adaptations, a performance-monitoring system in the posterior medial frontal cortex (pMFC) signals the need for adjustments (*11*, *12*). The rostral cingulate zone (RCZ), located in the pMFC, has been suggested to be involved in learning from errors (*13*, *14*). A neurobiological theory holds that this region receives dopaminergic teaching signals from the midbrain coding whether an event is better or worse than predicted (*14*). In close interaction with the performance-monitoring system, the basal ganglia, in particular the nucleus accumbens (NAC), play a major role in reward-based learning (*12*, *15*–*17*). Moreover, the performance-monitoring system needs to interact with the hippocampal formation to enable learning of stimulus-reward associations.

To investigate neural activity related to error-based learning, we recorded functional magnetic resonance imaging (fMRI) data from 26 healthy male subjects grouped by genotype [A1-allele carrier, A1+ group, $n = 12$; non–A1-allele carrier, A1– group, $n = 14$ (*18*)]. We used a probabilistic learning task sensitive to dopaminergic manipulations (*2*). Participants had to learn to choose the more-often rewarded symbols from pairs of stimuli presented in random order. After each choice, probabilistic feedback was provided (Fig. 1, top). After learning, participants were confronted in a behavioral posttest with the same symbols, now paired with symbols other than the one from the learning phase [supporting online material (SOM), table S1]. This allowed us to disentangle preference for the most-often rewarded symbol "A" and avoidance of the least-often rewarded symbol "B."

The groups defined by the presence or the absence of the A1 allele did not differ in the average frequency of selecting favorable symbols nor in the rate of negative feedback; however, we found a remarkable group difference in avoidance learning (Fig. 1, bottom left) (SOM text). In the posttest, the A1+ group avoided the negative symbol B significantly less than they chose the positive symbol A ($P = 0.03$). Moreover, their avoidance of B was reduced compared with the A1– group ($P = 0.03$), who did not show a significant difference between selecting A and avoiding symbol B ($P = 0.17$). Consistent with this behavior, they also showed a reduced negative feedback–related fMRI signal in the RCZ ($x = 4$, $y = 24$, $z = 33$; z score = 3.5, 324 mm^3) compared with the A1– group (Fig. 2A, and table S2). In a Bayesian analysis (*18*, *19*), we observed a posterior probability of 95.8% for a group difference in RCZ activity induced by negative feedback. Moreover, only members of the A1– group showed positive correlations with negative feedback–related RCZ activity and preference for the A symbol ($r = 0.53$, $P = 0.05$) and avoidance of the B symbol ($r = 0.55$, $P = 0.04$). A further strong signal increase on negative feedback in the right middorsal prefrontal cortex [$x = 40$, $y = 21$, $z = 27$; z score = 4.3, middle frontal gyrus (MFG)] was found only in the A1– group (posterior probability of group difference: 97.1%).

To study learning over the course of the probabilistic learning task, we modeled subjects' behavior using a modified Rescorla-Wagner reinforcement learning model (*20*) (fig. S1). In this computational model, the difference in activity of the output neurons provides a trial-by-trial estimate of certainty of the given response. The A1– group reached a significantly higher response certainty in the last third ($t = 2.2$, $P = 0.04$). The development of the certainty over the course of the experiment is shown in Fig. 1 (bottom right). In both groups, the curves resemble a logarithmic learning curve with a steep increase in the first third and an asymptotic course at the end of the experiment. After an initial period of about 200 trials, the A1– group developed a higher response certainty than the A1+ group. For both genetic groups, response certainty negatively correlated with pMFC activity (fig. S2). Note that, in the A1– group, the time course of certainty, which reflected learning progress, showed a positive correlation with activity in the posterior hippocampus bilaterally ($x = 22$, $y = –39$, $z = 6$; z score = 3.9, 216 mm^3 and $x = –23$, $y = –39$, $z = 3$; z score = 3.5, 81 mm^3), whereas no such correlation was found in A1+ participants [Bayesian posterior probability of group difference: right, 94.9%; left, 96.2% (Fig. 2B) and table S3].

How does feedback monitoring in the RCZ interact with forming memories in the hippocampus? Anatomically, these areas are connected via the cingulate bundle. To investigate learning-related changes in functional interactions of the RCZ and other brain areas over time, we performed a psychophysiological interaction analysis (PPI) (*21*). The experiment was divided into three parts of equal length. We then contrasted the functional connectivity of the RCZ observed in the first third with the connectivity observed in the last third of the learning experiment, thereby capturing the difference between steep rule acquisition in the beginning and more stable rule exploitation at the end. Again, in the A1– group, we observed a significant change over time: In the first third of the experiment, the functional coupling between the RCZ activity and the bilateral hippocampus was substantially stronger

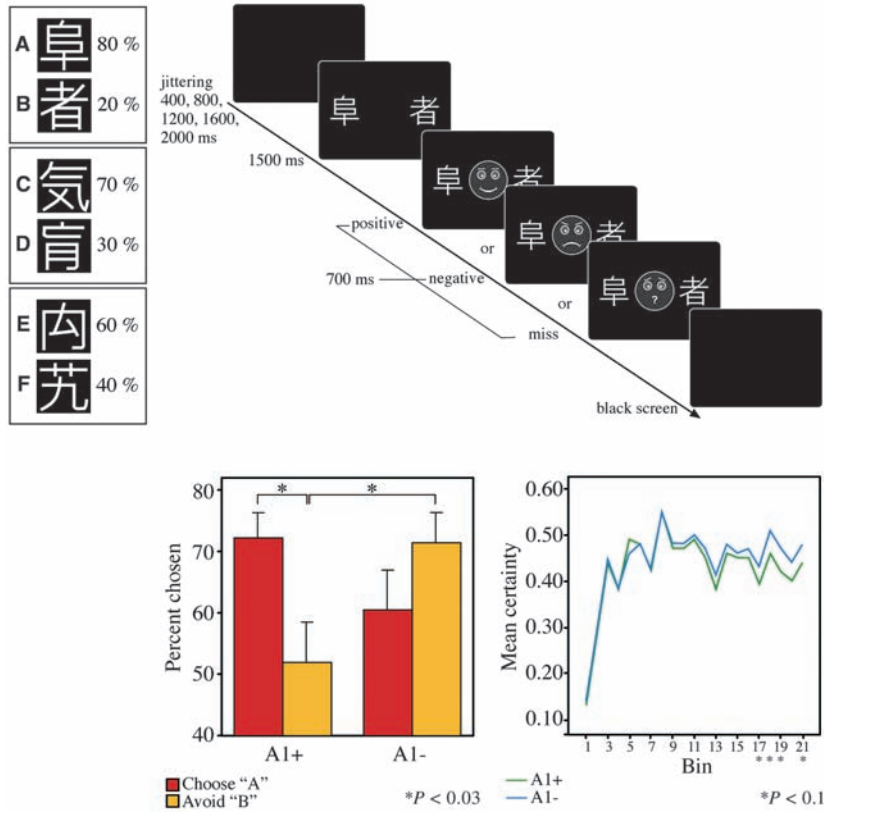

Fig. 1. Probabilistic learning task, behavioral and computational results. (Top) Stimuli, reward probabilities (percent positive feedback), and schematic trial sequence of the probabilistic learning task (*2*). (Bottom, left) Result of the behavioral post test: Choosing the good symbol (A) and avoiding the bad one (B) differs between the two genetic groups (group × selection interaction: $F_{1,24} = 8.1$, $P = 0.009$). (Bottom, right) Certainty of the given response resulting from the computational model, binned into bins of 20 trials each and differentiated between the two genetic groups.

than in the last third (Fig. 2C). The A1+ group showed no such correlation (Bayesian posterior probability of group difference: left hippocampus, 99.98%; right hippocampus, 99.91%). Furthermore, only the A1– group showed a similar change in functional coupling between NAC and RCZ over the time course of the experiment (Bayesian posterior probability: 99.54%). The NAC, another major target of dopaminergic projections, has also been implicated in feedback-based decision-making (*12*, *22*–*24*). The fMRI signal in the NAC on both sides was increased by positive feedback as compared with negative feedback (Fig. 2A). This reward-related activity increase was reduced in the A1+ group in the right NAC ($x = 16$, $y = 9$, $z = -6$; z score = –3.96; Bayesian posterior probability of group difference: 94.8%; on the left side, posterior probability reached only 74.1%).

Taken together, our results confirm that dopamine plays a major role in performance monitoring and behavioral modification for reaching optimal performance levels: Alterations in dopaminergic transmission lead to corresponding alterations in negative feedback processing and, related to this, to differences in learning from negative feedback. It appears that reduced dopamine D2 receptor density is associated with reduced capacity to learn negative characteristics of a stimulus from negative feedback. High receptor density in the A1– group is associated with clear avoidance of the most negative stimulus, whereas a reduced receptor density in A1+ subjects is not. Corresponding to this, subjects with a reduced receptor density show a weaker blood oxygen level–dependent (BOLD) response to negative feedback in the performance-monitoring network consisting of the pMFC and basal ganglia. In the pMFC, this difference was specific to negative feedback; its response to positive feedback and negative correlation with certainty (*12*) did not differ between groups (Fig. 2A). Negative feedback–related pMFC activity predicted posttest performance in the A1– group, which suggests that they used negative feedback for avoidance, as well as preference learning. Interestingly, anterior insular activity, thought to be involved in autonomic responses to errors (*12*), was present in both groups, which suggests that the genotype-effect is specific to learning from errors. The differential activity in the MFG, a brain region commonly found in working memory tasks (*25*), may suggest that A1– participants used a monitoring-within-memory strategy of keeping track with selection outcome history. This speculation is supported by the role of prefrontal D2 receptors in working memory functions (*26*, *27*).

Hence, the genetically driven differences in avoidance learning seem to result from a weaker neuronal response to negative feedback. Reduced monitoring signals are less likely to influence the memory system. This is supported by the finding of a reduced interaction of performance monitoring in pMFC and memory-formation in the hippocampus.

It is noteworthy that the fMRI signal reduction in the A1– group is specific to performance monitoring–related processes. It does not generalize to other task-specific activity (SOM text and fig. S4).

At first sight, our findings that subjects with lower D2-receptor densities show reduced avoidance learning may appear to conflict with results indicating that patients with Parkinson's disease on medication, i.e., with enhanced dopaminergic transmission, have problems in learning the negative value of stimuli (*2*). This apparent discrepancy can be resolved by a recent study, which revealed a higher rate of dopamine synthesis in the striatum for subjects with the A1+ configuration compared with A1– subjects (*28*). A reduction in D2 receptors could also affect D2 autoreceptors, which in turn leads to a higher synthesis rate of dopamine. Accordingly, transmission via the unaffected D1 receptors should be strengthened, whereas modulation of phasic postsynaptic D2 activity should be relatively reduced. This should lead to a relative decrease in avoidance learning and a shift to learning mainly from positive reinforcement (*2*, *5*). Parkinson's disease is often treated with tonically acting direct D2 agonists, which also reduce phasic modulations at postsynaptic D2 receptors. A phasic decrease in dopamine, as is suggested to occur after negative feedback (*4*, *14*), may thus be less effective in both studies. This dulled D2-mediated dopaminergic signal in turn would finally lead to a weaker hemodynamic response in the RCZ.

Many studies have found relations between a reduced dopamine D2 receptor density and addiction, obesity, or compulsive gambling (*9*, *10*, *29*). It may be speculated that the insensitivity to negative consequences of an action, as described above, is one feature of a low D2

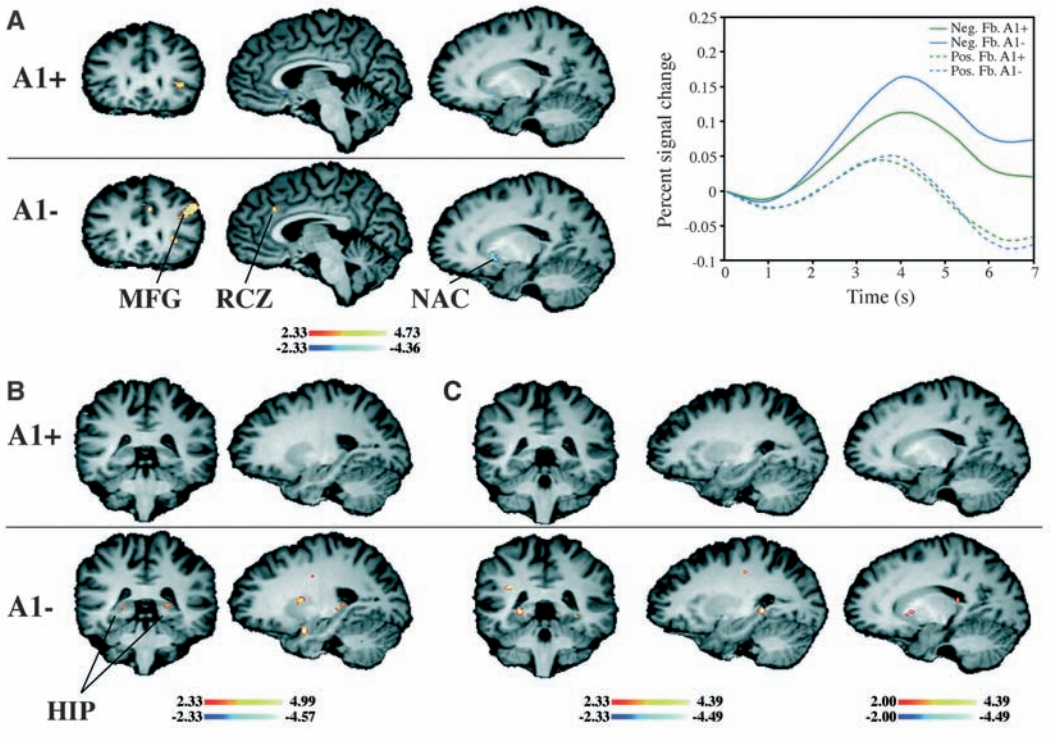

Fig. 2. Genetic influences on the fMRI results. Only clusters with at least 81 mm^3 activated at $z \geq 3.09$ are shown. For visualization, the map thresholds are set at $z = 2.33$ (unless stated otherwise). (**A**) (Left) The contrast between negative and positive feedback for the two genetic groups is shown projected onto a coronal slice ($y = 24$) and two sagittal slices ($x = 4$ and $x = 16$); red, negative feedback > positive feedback; blue, positive feedback > negative feedback. (Right) Percent signal change for positive and negative feedback taken from RCZ ($x = 4$, $y = 24$, $z = 33$). (**B**) Parametric within subject fMRI analysis using the certainty of the given response as a regressor, projected onto a coronal ($y = -39$) and a sagittal ($x = 22$) slice. HIP, hippocampus. (**C**) Psychophysiological interaction analysis between RCZ ($x = 4$, $y = 24$, $z = 33$) and other brain areas, projected onto a coronal ($y = -42$) and two sagittal ($x = -26$ and $x = 16$) slices. Red, stronger interaction in the first third than in the last third of the experiment; blue, stronger interaction in the last than in the first third.

receptor configuration and promotes behavior that could threaten health or social interactions.

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Supporting Online Material

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Ketamine-Induced Loss of Phenotype of Fast-Spiking Interneurons Is Mediated by NADPH-Oxidase

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Abuse of the dissociative anesthetic ketamine can lead to a syndrome indistinguishable from schizophrenia. In animals, repetitive exposure to this *N*-methyl-D-aspartate–receptor antagonist induces the dysfunction of a subset of cortical fast-spiking inhibitory interneurons, with loss of expression of parvalbumin and the γ-aminobutyric acid–producing enzyme GAD67. We show here that exposure of mice to ketamine induced a persistent increase in brain superoxide due to activation in neurons of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Decreasing superoxide production prevented the effects of ketamine on inhibitory interneurons in the prefrontal cortex. These results suggest that NADPH oxidase may represent a novel target for the treatment of ketamine-induced psychosis.

The *N*-methyl-D-aspartate (NMDA)–receptor (NMDA-R) hypofunction theory of schizophrenia proposes that the effects of NMDA-R antagonists, such as phencyclidine (PCP) and ketamine, produce symptoms of schizophrenia in healthy humans because of specific effects on inhibitory circuits that lead to disinhibition of neurotransmitter systems (*1*). Disinhibition of glutamatergic activity, resulting in increased excitatory transmission, was confirmed in the prefrontal cortex (PFC) of rodents and nonhuman primates (*2*). However, after prolonged exposure, the increased excitatory neurotransmission is followed by a depression of brain activity (*3*) that occurs through an unknown mechanism.

Derangements of γ-aminobutyric acid (GABA)–mediated systems in schizophrenia have been consistently observed in postmortem tissue (*4*). Initial in situ hybridization studies showed reduced expression of GAD67, the main isoform synthesizing GABA in brain (*5*). Subsequent studies showed also that the expression of the calcium-binding protein parvalbumin (PV) was reduced in postmortem samples (*6*, *7*). Finally, NMDA-R antagonists also induce a decrease in PV expression (*8*, *9*). This apparent "loss of GABAergic phenotype" in PV-containing interneurons led to the suggestion that dysfunction of these fast-spiking inhibitory interneurons may be a core feature of the disease (*10*).

PV interneurons are involved in the generation of gamma oscillations responsible for temporal-encoding and storage or recall of information required for working memory (*11*). These interneurons receive the largest glutamatergic input among all GABA-releasing neurons in cortex (*12*) and are highly sensitive to NMDA-R antagonists (*13*), a feature that may be related to the role played by NMDA-Rs in the control of basal synaptic activation in these interneurons (*14*).

We previously showed that primary cortical neuronal cultures respond to NMDA-R antagonists with a reversible loss of GAD67 and PV in PV interneurons (*15*). These neuronal cultures contain about 10 to 20% GABAergic neurons, of which 50% are PV interneurons (*15*), and show spontaneous glutamatergic and GABAergic activity (*16*, *17*). We hypothesized that if the initial disinhibition of excitatory transmission produced by NMDA-R antagonists observed in vivo also occurred in cultured cortical neurons, then bypassing the need for GABA production by adding a γ-aminobutyric acid type A $GABA_A$ agonist should prevent NMDA-R antagonist–mediated effects (*18*). Exposure to the GABA agonist muscimol prevented ketamine-mediated decrease in PV and GAD67 in PV interneurons (Fig. 1 and fig. S1), which suggested that loss of an inhibitory input to excitatory neurons, the main neuronal subpopulation in these cultures, is involved in the subsequent loss of phenotype of PV interneurons.

A rapid increase in reactive oxygen species (ROS) occurs in vitro (*19*), and in vivo (*20*) after exposure to NMDA-R antagonists, which indicates increased oxidative stress. However, what mechanism initiates this increase is not clear. The recent demonstration of expression of the superoxide-producing enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in hippocampus (*21*) led us to test the possibility that disinhibition of neurotransmission by NMDA-R antagonists leads to increased NADPH oxidase activity. We measured the oxidation product of dihydroethidium (DHE) by confocal microscopy and analyzed the levels of superoxide production in cultured neurons after prolonged exposure to low concentrations of ketamine. A significant increase in neuronal superoxide production was observed after 24 hours' exposure to 0.5 μM ketamine, which was prevented by muscimol (Fig. 1). The increase in superoxide in response to ketamine was not restricted to PV interneurons (Fig. 1B), which suggested that activation of the enzyme(s) producing superoxide occurs throughout cortical neurons. We next determined whether the increase in superoxide was involved in the loss of GABAergic phe-

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