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Disruption of Interneuron Development

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Summary: Disruption of gamma-aminobutyric acid (GABAergic) interneuron development during the embryonic and early postnatal periods can have profound neurological and behavioral consequences. Hepatocyte growth factor/scatter factor (HGF/SF) has been identified as an important molecular cue that may guide the movement of interneurons from their birthplace in the ganglionic eminences (GE) to their final resting place in the neocortex. In vitro studies demonstrate that decreased HGF/SF bioactivity in pallial and subpallial tissues is associated with a reduction in the number of cells migrating out of GE explants. The uPAR knockout mouse provides a unique opportunity to study the effects of interneuron disruption in vivo. uPAR$^{-/-}$ mice have reduced HGF/SF bioactivity in the GE during the period of interneuron development and a concomitant 50% reduction in the number of GABAergic interneurons seeding frontal and parietal regions of the cerebral cortex. Behaviorally, these mice display an increased susceptibility to seizures, heightened anxiety, and diminished social interaction. This article discusses the commonalities between the functional defects seen in uPAR$^{-/-}$ mice and those of humans with developmental disorders, such as epilepsy, schizophrenia, and autism. It is suggested that disruption of GABAergic interneuron development may represent a common point of convergence underlying the etiologies of many of these developmental disorders. **Key Words:** Interneuron development—Hepatocyte growth factor—Neocortex.

Experimental and clinical data suggest that epilepsy is caused by an imbalance between excitatory and inhibitory neurochemical systems in the brain and that gamma-aminobutyric acid (GABAergic) interneurons play a critical role in maintaining this balance. By acting as a sensory gate, inhibitory GABAergic interneurons regulate the degree of glutaminergic excitation in the neocortex, filtering the input, and coordinating the output of multiple projection neurons. The fact that an approximate 6:1 ratio of glutamatergic neurons to GABAergic interneurons in the neocortex is consistently observed across mammalian species suggests that this ratio may represent an important numerical balance for normal brain function and behavior. GABAergic interneurons comprise only a small subfraction of cells in the neocortex. Nonetheless, disruption of their development, and hence the delicate balance between excitation and inhibition, may have profound neuropsychiatric repercussions. In addition to epilepsy, developmental interneuron perturbations have been linked to autism (1,2) and schizophrenia (3), two other disorders that often emerge during childhood and adolescence (4–6).

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INTERNEURON DEVELOPMENT

Brain maturation is an extremely well-orchestrated process consisting of multiple steps to ensure that targeted cells are generated with specific phenotypic properties in an orderly fashion. Historically, it was thought that all neocortical cell populations arose from the dorsal telencephalic proliferative zones and migrated radially to their cortical destinations. There is now convincing evidence, however, that separate pools of progenitor cells in distinct regions of the brain give rise to different types of neurons, and that the function of these neurons is determined by their final resting place after migration (7). For example, excitatory glutamatergic neurons are born in the proliferative zone of the dorsal pallium, the forerunner of the neocortex, and migrate radially to form the cortical plate (8). The majority of the inhibitory GABAergic neurons, on the other hand, originate in the ganglionic eminences (GE) of the subpallial telencephalon, which is the forerunner of the basal ganglia (9). From here, they either migrate tangentially to the neocortex where they become integrated into the cortical circuitry as interneurons or remain in

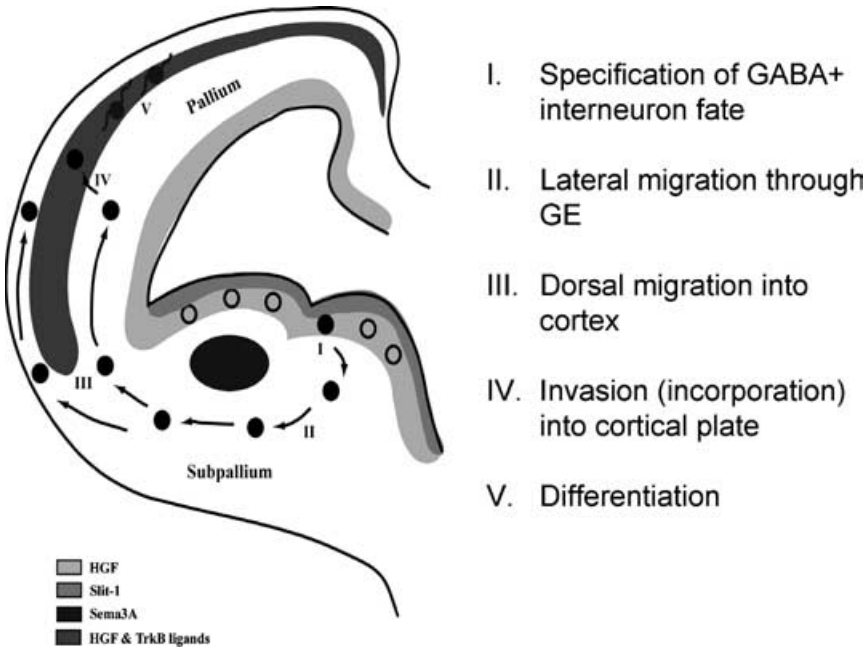

FIG. 1. Stages of cortical interneuron development. Most GABA interneurons are born in the subpallium (i), and migrate laterally through the ganglionic eminence (ii) and then dorsally into the overlying cortex (iii) where they become incorporated into the laminar structure (iv and v) [from Levitt et al., 2004 (2)]. Reprinted from *Trends Neurosci* Copyright 2004, with permission from Elsevier.

the basal ganglia where they differentiate into projection neurons.

Evidence that GABAergic interneurons migrate tangentially, rather than radially, came from two independent laboratories. Using dye labeling, de Carlos and colleagues (10) were the first to demonstrate that a subpopulation of progenitor cells in the subpallial telencephalon migrate dorsally from the GE to the overlying neocortex. This migratory path is shown in Fig. 1. The subpallial site of origin and identification of the cells as GABAergic were then confirmed by Anderson (9) using the Dlx1/Dlx2 mutant mouse. Compound mutations of the Dlx genes, which are expressed exclusively in the ventral subpallium during fetal development, disrupt progenitor pools and cause an 80% reduction in the final number of interneurons in the adult mouse neocortex. Additional work has revealed that there are three structurally different regions of the GE, the medial, lateral, and caudal domains, each of which gives rise to distinct groups of interneurons. In the mouse, the majority of cortical interneurons arise from the medial GE early in neurogenesis, whereas the lateral GE contributes a smaller pool of later-developing interneurons that eventually populate the olfactory bulb and striatum (11).

The brain is most vulnerable to disruption during the prenatal and early postnatal periods when there is rapid growth and differentiation of neuronal populations. It is during this time that cells are born, migrate to their distant destinations, and integrate into the local circuitry of specific lamina of the neocortex. There are three major steps or decision points in the migration process. At each decision point, the developmental or functional path of the interneuron is regulated by specific signaling molecules that are uniquely susceptible to disruption:

- First, the production of GABAergic neurons; progenitor cells in different regions of the GE differentiate into different populations of neurons.
- Second, the choice of migratory route (ventral, lateral, or dorsal); the route selected will determine the final destination of the GABAergic neurons.
- Third, the process of integration into the local circuitry of the developing cortex and the establishment of the correct balance between excitatory and inhibitory neurochemical systems.

The molecular cues that guide the movement of interneurons at each decision point are not well understood. Convergent evidence suggests that multiple cues are involved, including repulsive/attractive cues that force cells to change their course of direction during migration and growth factors, such as brain-derived neurotrophic factor (BDNF), neurotrophin 4 (NT4), and Hepatocyte growth factor/scatter factor (HGF/SF), which induce the motility of cells (12–15). The discovery that HGF/SF plays a significant role in cortical development was surprising, given its well-known participation in the biochemical pathways mediating thrombolysis, angiogenesis, and metastasis. Using a variety of techniques, including immunohistochemistry, in situ hybridization, dye-labeling, and cultured embryonic forebrain explants, Powell and colleagues (13) demonstrated that the bioactivity of HGF/SF increases markedly in both pallial and subpallial tissues during the period of interneuron development and is

associated with a simultaneous increase in the motility and migration of cells out of the GE. Altering HGF/SF activity, through the administration of exogenous HGF or by adding a blocking anti-HGF antibody to the culture media, disrupts the orderly migration of cells out of GE explants and induces undirected scatter, thus reducing the number of cells reaching the cortex. Taken together, these data indicate that HGF/SF plays a critical role in promoting the migration of interneurons during early development.

THE uPAR$^{-/-}$ MOUSE AS A DEVELOPMENTAL MODEL OF INTERNEURON DISRUPTION

Attempts to confirm HGF/SF's role in interneuron development in vivo were initially frustrating because single target deletions of either the HGF or Met receptor genes, which control HGF activity in the mouse, were found to cause midgestation lethality before the onset of interneuron migration (16,17). In contrast, mice carrying a deletion for the gene encoding urokinase-type-plasminogen-activator receptor (uPAR), one of the factors that cleaves the inactive pro-form of HGF/SF to a biologically active protein, are viable and fertile (13,18). uPAR$^{-/-}$ knockout mice have diminished levels of HGF/SF and C-Met protein expression and decreased HGF/SF bioactivity in the pallium and subpallium during the period of interneuron migration. Since the uPAR$^{-/-}$ mouse survives into adulthood, it is an ideal model for the study of the long-term behavioral consequences of developmental perturbations in the maturation of GABAergic interneurons.

The uPAR$^{-/-}$ mouse, on a mixed C57/129SeV strain background, has no obvious phenotype. The fact that no gross cortical abnormalities are revealed by traditional Nissl staining is not surprising, given the small number of GABAergic interneurons relative to glutaminergic projection neurons and glia in the cerebral cortex of adult mice (13). More sensitive quantification techniques, however, reveal that uPAR$^{-/-}$ mice have a significant loss of cortical GABAergic interneurons. Using immunoreactive calbindin protein as a marker of migrating interneurons, a 50% reduction in GABAergic interneurons was documented in the cortices of uPAR$^{-/-}$ mice on embryonic day 16.5 (E16.5) and postnatal day 0 (P0) (18). By far the most interesting phenotypic feature of disrupted interneuron development is, however, its spatially selective nature, which suggests that cell migration from the medial GE is preferentially disrupted in uPAR$^{-/-}$ mice. As shown in Fig. 2, the loss of immunoreactive GABA^{+} cells is restricted to the frontal (cingulate) and parietal cortices, leaving the more ventral piriform and posterior visual cortices relatively unaffected. Further analysis of those cortical regions containing a 50% reduction in GABA^{+}

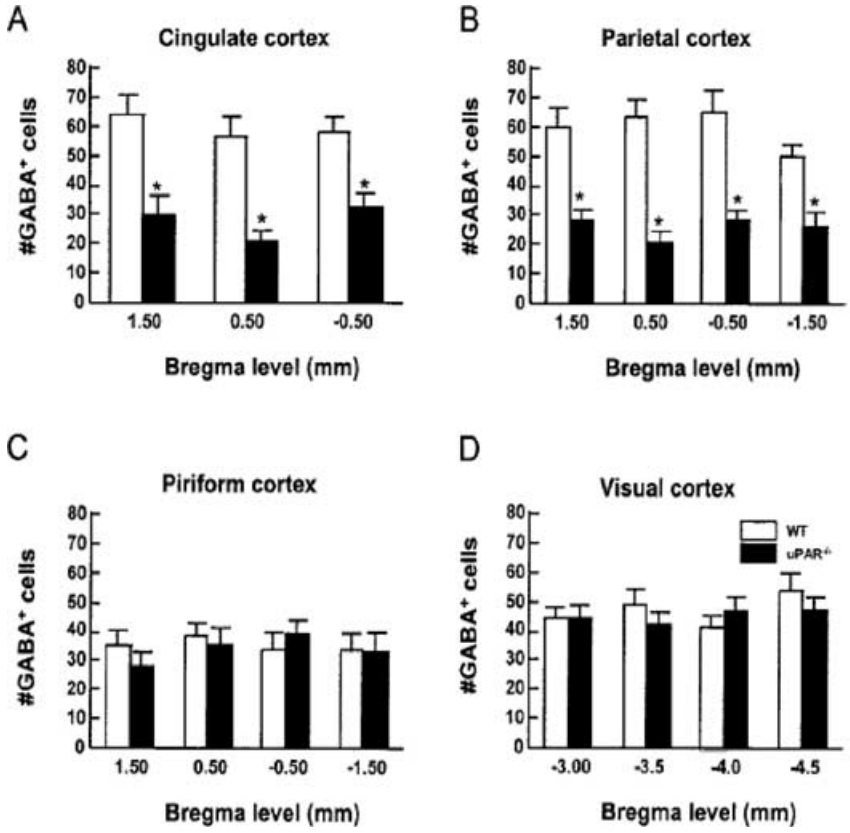

FIG. 2. The number of GABA interneurons in various regions of the cerebral cortex of the uPAR$^{-/-}$ mouse in comparison to WT controls. Data are presented as mean ± SEM. Asterisks denote a significant difference between WT and uPAR$^{-/-}$ mouse brains ($p < 0.05$). [from Powell et al., 2003 (18)]. Copyright 2003 by the *Soc Neurosci*.

cells using immunocytochemical techniques revealed that >90% of the lost interneurons were of the parvalbumin (PV^+) subtype (18). Both the calretinin and somatostatin subtypes were unaffected. Longitudinal studies comparing cell counts in infant and adult mice indicate that the spatially selective loss of interneurons is permanent (18).

In summary, $uPAR^{-/-}$ mice exhibit a complex phenotype characterized by a regionally specific disruption of interneuron development that selectively affects the PV^+ subtype within these regions. The fact that there is a 50% decrease in the number of GABAergic interneurons in the forebrain, an area intimately involved in the mediation of epilepsy and emotional behavior, suggests that $uPAR^{-/-}$ mice should display an array of concomitant behavioral abnormalities related to the loss of GABAergic inhibition.

One interesting additional finding arose in recent work examining the effect of the uPAR mutation when placed on the genetic background of a pure $C57Bl6^J$ congenic strain. Here, the approximate 50% reduction of GABAergic neurons and regional specificity (frontal and parietal only) are also exhibited, but there is only a partial reduction in the normal number of parvalbumin+ cells in these regions (19). These data indicate that the expression of certain phenotypic properties of GABAergic neurons, such as the expression of neuropeptides, may be controlled in a complex fashion by modifier genetic loci that regulate the impact of the uPAR mutation, or by patterns of physiological activity that differ between different mouse strains. This is not uncommon, as other examples of single gene mutations producing different phenotypes, depending upon background strain, have been reported (20,21).

INTERNEURON-DEFICIENT $uPAR^{-/-}$ MICE DISPLAY ENHANCED SEIZURE SUSCEPTIBILITY

Approximately 6% of $uPAR^{-/-}$ mice (vs. 0% of WT) exhibit spontaneous seizures, and occasionally these are lethal. Electroencephalographic (EEG) recordings, obtained from freely moving $uPAR^{-/-}$ and WT mice during 24-h video/electrographic monitoring, are presented in Fig. 3 (18). As shown in Part A, $uPAR^{-/-}$ mice characteristically display periods of low amplitude, desynchronized EEG during waking periods. Mutant mice also exhibit frequent abnormal slow waves and interictal discharges. A recording of a spontaneous bilateral tonic clonic seizure lasting about 75 s is presented in Part B of the figure. This illustrates a typical example of the buildup of periodic interictal discharges and a rather abrupt initiation of high-voltage, rhythmic spike, and spike-wave seizure discharges. The discharges in the $uPAR^{-/-}$ mice usually terminated abruptly, with no postictal depression. As

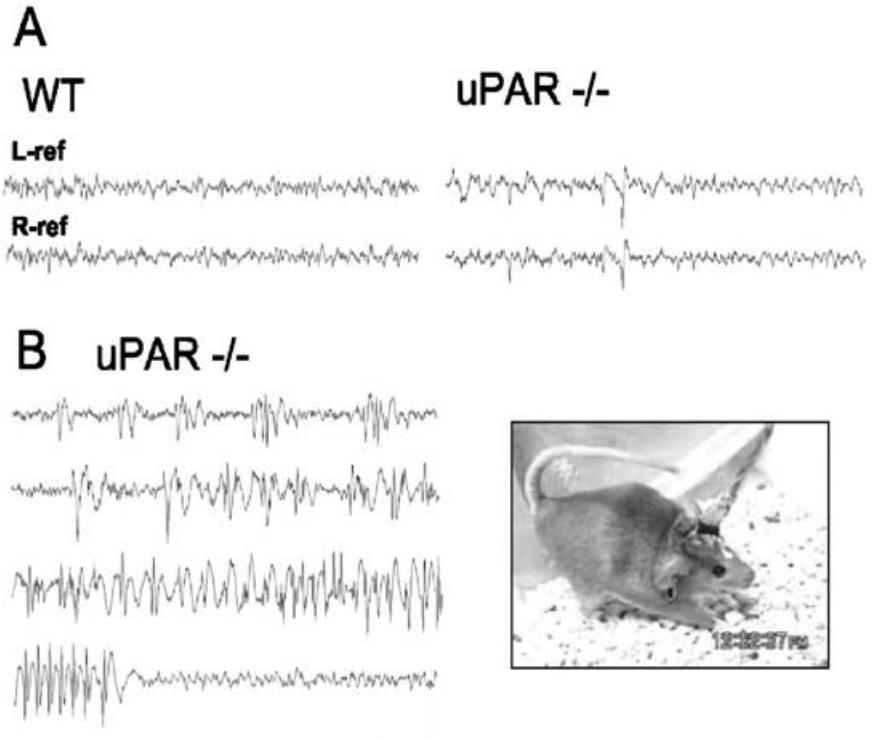

FIG. 3. EEG recordings from freely moving WT and $uPAR^{-/-}$ mice. **A:** Baseline EEG recordings in an awake $uPAR^{-/-}$ mouse reveal abnormal bilaterally synchronous discharges. **B:** A recording of a spontaneous bilateral tonic clonic seizure lasting about 75 s. The picture inset on the right shows the typical posture displayed by a $uPAR^{-/-}$ mouse during the final stage of a seizure [from Powell et al., 2003 (18)]. Copyright 2003 by the *Soc Neurosci*.

expected for these types of seizures, the mice maintained a tonic posture of flexion during the EEG seizure, exhibiting intermittent truncal movements, bilateral clonic forelimb movements, and Straub tail.

uPAR$^{-/-}$ mice are also more susceptible to the convulsant effects of the GABA antagonist, pentylenetertrazol (PTZ). In a recent publication, 100% of uPAR$^{-/-}$ mice reportedly developed full tonic clonic seizures following a single threshold dose of 50 mg/kg (s.c.) PTZ, in comparison with only 50% of WT controls (18). In addition, the latency to first forelimb clonus was 67% faster in uPAR$^{-/-}$ mice (456 s vs. 681 s in WT controls) and the subsequent seizures were more severe. When rated on a 3-point scale, 90% of uPAR$^{-/-}$ mice exhibited grade three seizures (tonic extensions or death), compared with 0% of their WT littermates.

The increased susceptibility of uPAR$^{-/-}$ mice to seizures is consistent with a 50% decrease in cortical GABAergic interneurons and diminished inhibitory control of excitatory neurotransmitter systems. The fact that uPAR$^{-/-}$ mice also show an increased sensitivity to low-dose diazepam (0.1–1.5 mg/kg), as indicated by a dose-dependent reduction in locomotor activity relative to WT mice (E. Powell and P. Levitt, unpublished data), suggests that there may be simultaneous changes in the number and/or sensitivity of benzodiazepine receptors to compensate for the loss of GABAergic tone.

uPAR$^{-/-}$ MICE DISPLAY INCREASED ANXIETY AND IMPAIRED SOCIAL INTERACTION

uPAR$^{-/-}$ mice consistently display behaviors indicative of increased anxiety in three behavioral paradigms: the open field, light-dark exploration, and elevated plus maze (18). In each of these paradigms, mice are presented with a conflict between their natural drive to explore novel places and their fear of open or brightly lit spaces. With time, normal mice will overcome their fear and begin to explore the open spaces. Failure to explore or reduced exploratory activity relative to normal controls is interpreted as increased anxiety (22,23).

uPAR$^{-/-}$ and WT mice are equally active in the open-field, in that they traverse similar distances during test periods. However, uPAR$^{-/-}$ mice spend significantly more time moving round the outer edges of the arena (within 6 cm of the wall) and significantly less time in the open center space than WT controls. Quantification of open-field test results revealed that uPAR$^{-/-}$ and WT mice spent 76% and 34%, respectively, of their time moving around the outer edges of the arena and 24% and 66% of their time in the center space ($p < 0.01$). Similar results have been obtained using both the classic light-dark avoidance test and the elevated plus maze paradigms (18). The graph in Fig. 4 depicts the time uPAR$^{-/-}$ mice spent in the closed and open arms of the elevated plus maze in

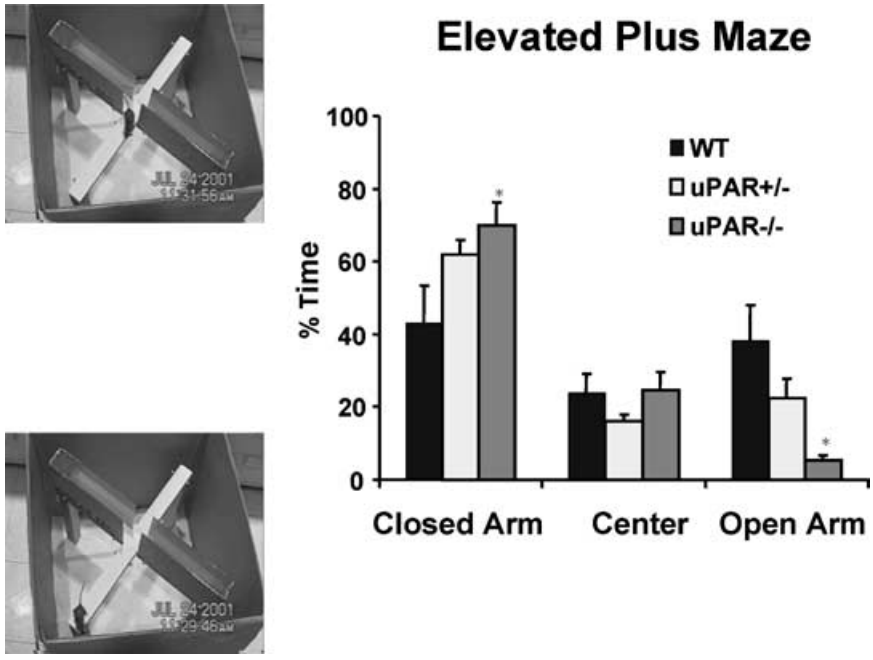

FIG. 4. The percentage of time uPAR$^{-/-}$ and WT mice spent in the enclosed (closed arm and center space) and open arms of the elevated plus maze. Asterisks indicate significant differences between groups ($p < 0.05$). Picture insets show the increased reluctance of uPAR$^{-/-}$ (top) to explore the distal ends of the open arms of the maze in comparison to WT controls (bottom) [from Powell et al., 2003 (18)]. Copyright 2003 by the *Soc Neurosci.*

comparison with WT controls. As shown, uPAR$^{-/-}$ and WT mice spent about the same amount of time exploring the enclosed portions of the maze (closed arm and center space). However, uPAR$^{-/-}$ mice spent significantly less time (<5%) in the brightly lit open arms than their littermate controls (35%). Furthermore, none of the uPAR$^{-/-}$ mice explored the most distal ends of the open arms. These behavioral observations are consistent with the interpretation that uPAR$^{-/-}$ mice display heightened anxiety in novel situations.

In addition to increased anxiety, uPAR$^{-/-}$ mice also exhibit impaired social behavior (E. Powell, B. Thompson, and P. Levitt, unpublished data), In the resident-intruder task, an unfamiliar (intruder) mouse is introduced into the home cage of another mouse. The pair of mice is then observed for a designated period of time to assess the degree and type of social interaction. In this paradigm, uPAR$^{-/-}$ mice spend about half as much time interacting with the intruder than WT controls.

COMMONALITIES BETWEEN THE uPAR$^{-/-}$ PHENOTYPE AND HUMAN DEVELOPMENTAL DISORDERS

A single mutation of the uPAR$^{-/-}$ gene results in a constellation of permanent neuroanatomical, neurochemical, and behavioral defects. The presence of these defects underscores the importance of the GABAergic interneuron circuitry in the maintenance of normal behavior and the challenge presented to the developing brain to override such specific defects through adaptation. uPAR$^{-/-}$ mice exhibit a complex phenotype characterized by a regionally specific disruption of interneuron development in the forebrain that selectively affects the PV^{+} interneuron subtype. The consequences of this neuropathology are, in turn, reflected in a number of concomitant behavioral deficits, including an increased susceptibility to seizures, heightened anxiety in novel situations, and impaired social behavior. These findings are consistent with the interpretation that early disruption of GABAergic interneuron development results in diminished inhibitory control of cortical excitatory neurocircuitry and an abnormal formation of cortical maps.

In addition to modeling epilepsy, the uPAR$^{-/-}$ mouse mutant provides a conceptual framework to link the developmental disruption of GABAergic neuron function with the pathophysiology of other human developmental disorders, such as schizophrenia and autism. One of the neuroanatomical hallmarks of schizophrenia is a selective decrease in the number of synapses arising from PV^{+}-containing interneurons in prefrontal regions of the brain with a sparing of both calretinin-containing and somatostatin-containing interneurons (24,25). Although the absolute number of interneurons is not as reduced in individuals with schizophrenia as it is in the uPAR$^{-/-}$ mouse, the functional consequences of reduced synaptic contact between inhibitory GABAergic interneurons and excitatory glutaminergic neurons may produce very similar behavioral effects. Common symptoms associated with schizophrenia include anxiety, cognitive dysfunction, and impaired socialization.

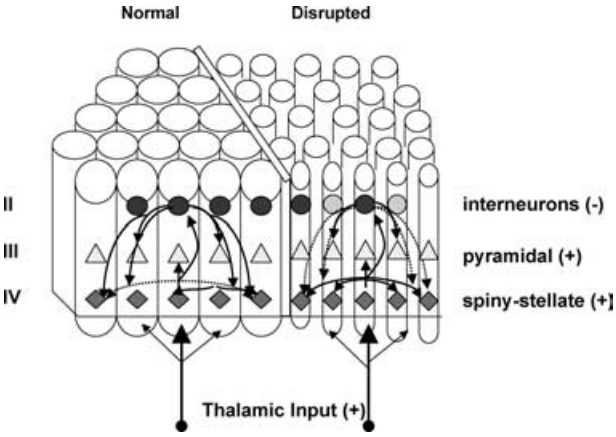

FIG. 5. Schematic comparing the proposed minicolumn structure in normal and autistic (disrupted) cortical tissue. Each minicolumn is a discrete unit consisting of excitatory and inhibitory elements that receive and integrate thalamic input. Reductions in the number of GABAergic interneurons would theoretically modify the quantitative and/or qualitative precision of the excitatory drive. II, II, IV, cortical layers; (–), inhibitory; (+), excitatory [adapted from Casanova et al., 2002 (26) and Levitt et al., 2004 (2)]. Reprinted from *Trends Neurosci*. Copyright 2004, with permission from Elsevier.

Although the neuropathology of childhood autism is not as well understood, at least one line of evidence suggests that regional disruption of interneuron development may underlie this disorder as well. Analysis of postmortem brains from subjects with autism indicates that cell minicolumns in the prefrontal cortex and temporal lobes, consisting of axon projections and terminations of double bouquet interneurons, are narrower and less densely packed with cells than those of control children (26). Fig. 5 shows a schematic comparing the proposed minicolumn structure in normal and autistic cortical tissue. Each minicolumn is a discrete unit consisting of excitatory and inhibitory elements that receive and integrate thalamic input. Reductions in the number of GABAergic interneurons would theoretically modify the quantitative and/or qualitative precision of the excitatory drive. Autism can be conceptualized as a disorder in which there is a functional defect in the modulation of arousal systems in the brain, which leads to a state of overarousal. It has been suggested that individuals with autism exhibit abnormal behaviors in an attempt to diminish or normalize arousal levels. In addition to disturbances in processing complex information, increased anxiety, and a tendency to avoid social interaction, many children with autism exhibit abnormal EEG patterns, and about 15–25% experience seizures (27). Although no rodent model can precisely phenocopy

a complex human disorder, the cortical neuropathology, presence of epilepsy, and behavioral deficits seen in individuals with autism mirror the disruptions exhibited by the uPAR$^{-/-}$ mouse, and thereby support the underlying role of altered GABAergic neurotransmission in the etiology of this disorder.

CONCLUSION

The uPAR$^{-/-}$ mutant mouse model demonstrates how a single gene mutation can have profound effects on the neuroanatomical and behavioral development of an organism. By changing the genetic background of the mouse, a vast array of phenotypic variations can be generated while leaving the core set of features related to the disruption of GABAergic interneuron development unchanged. Individuals with epilepsy, schizophrenia, and autism also display a heterogeneous array of phenotypic characteristics that apparently result from the complex interaction of genetic and environmental factors. Convergent evidence suggests that a developmental disruption of GABAergic interneurons may be a fundamental, core component of these disorders as well.

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