Archives Italiennes de Biologie, 144: 115-126, 2006.

SYNAPTICALLY-SILENT IMMATURE NEURONS SHOW GABA AND GLUTAMATE RECEPTOR-MEDIATED CURRENTS IN ADULT RAT DENTATE GYRUS

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INTRODUCTION

The hippocampus dentate gyrus (DG) retains neurogenic properties throughout adult life; this phenomenon has been extensively studied in rodent. Proliferating astrocyte-like precursors continuously generate new cells, some of which migrate, differentiate into mature neurons through a complex multi-step process and become part of neuronal networks (39, 43). Because of this phenomenon, neurons at different stages of morphological and functional maturation co-exist in the granule cell layer (GCL) of adult DG (3). In a previous investigation, we identified in the GCL of adult rat a population of highly immature neurons lacking functional synapses [classified as Class 1 (3)]. These neurons are located exclusively at the hilar border of GCL and represent the earliest stage of neuronal differentiation we have detected in adult rat GCL. The morphological and electrophysiological features of these synaptically-silent immature neurons appear similar to those of Type II late progenitors, which Fukuda *et al.* (15) showed to be at least up to 7-day-old in transgenic mouse GCL, and to those of 7-day-old immature neurons described by Esposito *et al.* (14) in female mice undergoing spontaneous motor activity. Together with data showing that most 12-15-day-old adult-generated neurons in mouse GCL receive synaptic input (34), these findings suggest that Class 1 cells are in their first week of life. In a previous study, we found that in adult rat the fate of new neurons is determined early (11), accordingly with the findings obtained in mice by other Authors (25). In that investigation we observed that several of the newly-generated cells die by apoptosis mainly within the first week after their birth (11). A hippocampus-dependent learning occurring during this period is able to rescue a larger number of new neurons in adult rat dentate gyrus, as we previously reported (2). The lack of functional synaptic contacts leads to hypothesize a paracrine mechanism underlying this rescue.

During rodent brain ontogenesis, GABA and glutamate modulate a wide range of developmental events, such as cell proliferation, migration, differentiation and survival, occurring prior to synapse formation (see for review 7, 10, 29, 31, 36); in accordance, a variety of neuronal precursors and neurons at an early stage of differentiation express functional

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GABA and glutamate receptors before synaptogenesis (10, 27, 29, 30, 36), and these receptors can be paracrinely activated (12, 36). Both GABA and glutamate have been shown to modulate neurogenic processes in rodent adult brain (8, 9, 40). In order for these neurotransmitters to paracrinely act on early-differentiating, adult-generated synaptically-silent neurons generated in adulthood, a necessary requisite is that these neurons must express functional receptors for GABA and glutamate. Recently, Esposito *et al.* (14) showed that in exercised adult female mice dentate gyrus, silent 7-day-old neurons do respond to GABA and glutamate local application, indicating the presence of functional receptors for these neurotransmitters on their membrane.

Species and strain differences have been shown regarding, for example, the expression and sensitivity to ligands of some neurotransmitter receptors (1, 20); moreover, species-specific differences can be detected in adult neurogenesis and this phenomenon results differently regulated in rats and mice (4, 5). Finally, male and female animals show sex-related differences also concerning new neuron production in adult hippocampus (17, 22), due to the changes in gonadal steroid hormone levels related to estrous cycle.

Taking into account the above considerations, we investigated whether in DG of adult male rat living under laboratory standard conditions, granule layer cells in a very early stage of their differentiation, i.e. when they are synaptically-silent [Class 1 cells; (3)], express functional GABA and glutamate receptors, as occurs in adult exercised female mice. Hence, the whole-cell configuration patch-clamp technique was used and these cells were challenged with puff applications of GABA or glutamate in acutely isolated hippocampal slices; moreover, ionotropic and metabotropic GABA and glutamate receptor agonists and antagonists were locally applied to assess whether non-contacted immature neurons express different types of functional GABA and glutamate receptors and what are the characteristics of the currents elicited by activating these receptors. In addition, the effect of GABA receptor activation on membrane potential, and the E_{Cl} and intracellular chloride concentration of these cells were evaluated using gramicidin-perforated patch-clamp recordings. Finally, in order to verify whether adult-generated, 1-week-old granule layer cells show electrophysiological and morphological features typical of Class 1 cells, a group of rats were stereotaxically injected by GFP-expressing retroviral vector and GFP-positive cells were electrophysiologically analyzed 7 days after injection.

METHODS

6-8-week-old Sprague-Dawley male rats (n = 25) were used in accordance with Italian laws regarding animal experimentation. Animals were housed two per standard-sized cage, where the access to food and water was free. Room temperature was kept at 21 ± 1 °C, humidity was 50 ± 5%, and the light/dark cycle was 12-12 h (light on at 7.00 a.m.). After anesthesia with ketamine (65 mg/kg b.w.), rats were killed by decapitation, brains were rapidly removed and 400 μm-thick hippocampal transversal slices were prepared as previously described (3); slices were recovered in oxygenated artificial cerebrospinal fluid (ACSF, containing in mM: 125 NaCl, 2.5 KCl, 1.3 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, 1.3 Na^+ ascorbate, 0.6 Na^+ pyruvate, 10 dextrose; pH = 7.4; 320 mOsm) at room temperature for at least 1 h and then transferred to a recording chamber where they were continuously superfused with the same solution. Patch-clamp recordings were carried out in whole-cell or perforated configuration under visual guidance using a Zeiss Axioskop microscope (Carl Zeiss International, Italy) equipped with an infrared light-sensitive camera connected to a

monitor. For whole-cell configuration, recording pipettes were filled with the standard internal solution containing in mM: 126 potassium gluconate, 8 NaCl, 0.2 EGTA, 10 HEPES, 3 Mg_2ATP, 0.3 GTP (pH = 7.2; 290 mOsm). For perforated patch-clamp, the internal solution contained in mM: 134 KCl, 0.2 EGTA, 10 HEPES, and 5 μg/ml gramicidin previously dissolved in methanol (5 mg/ml); pipette tips were prefilled with gramicidin-free solution. Only highly immature granule cells belonging to Class 1, according to the previously reported classification (3), were considered; these cells, located at the hilar border of GCL, did not show synaptic input evoked stimulating either medial perforant pathway or granule cell layer and lacked spontaneous activity. They displayed depolarized resting membrane potential (RMP), high input resistance (IR) and low capacitance (C) consistent with their small size. In addition, they showed voltage-gated K^+ channels, while only 50% of them exhibited voltage-gated Na^+ channels, giving rise to a rudimentary spike. No currents mediated by voltage-gated Ca^{2+} channels were recordable in these cells. In order to detect and characterize GABA and glutamate receptor-mediated currents, specific drugs diluted in ACSF (100 μM GABA, 30 μM muscimol, 10 μM baclofen, 100 μM CACA, 100 μM glutamate, 300 μM NMDA, 100 μM Kainate, 100 μM ACPD; all drugs were purchased from Sigma, Italy) were locally applied by a computer-controlled pressure ejection system (homemade). The pressure ejection pipette was a multi-barreled tube, with 2-4 μm tip diameter, placed 10-15 μm from the analyzed cell body. The applied pressure was 7 bars and the drug application lasted 14 s (in whole-cell patch-clamp recordings) or 2 s (in patch-perforated recordings). Specific antagonists (10-100 μM bicuculline methiodide, 100 μM TPMPA, 200 μM saclofen, 300 μM furosemide, 50 μM AP 5, 30 μM CNQX, 500 μM MCPG; Sigma, Italy) were added either to the bath perfusion or the solution filling the tube. The GABA and glutamate receptor-mediated currents were recorded in voltage-clamp mode, if not otherwise specified. Voltages were corrected taking into account the junction potentials.

Green Fluorescent Protein (GFP) cDNA sequence was cloned in the Pinco retroviral expression vector (18). In the resulting construct, GFP-expression is controlled by a cytomegalovirus promoter. The packaging Phoenix cells (ORBIGEN) were transfected by the calcium-phosphate/chloroquine method (18) generating viral titers as high as 10^7 colony-forming units/ml. The supernatant containing the viral particles was collected after 48 hours, filtered, and stocked at - 80 °C until use. Retroviral stock carrying the GFP transgene was stereotaxically infused (twice 30 min spaced) into the dentate gyrus (1 μl at 0.5 μl per min) (anteroposterior = - 3 mm from bregma; lateral = 2 mm; ventral = 3.2 mm) of rats (6-7-week-old) anesthetized with sodium tiopenthal (45 mg/kg b.w.). Seven days after infusion, rats were killed and brain slices were obtained as described above. Recordings by patch-clamp in whole-cell configuration were carried out in GFP-positive cells; the presence of green fluorescence in the patch pipette after cell attached was verified before recording. Puff applications of GABA (100 μM) and glutamate (100 μM) were performed as reported above. Biocytin added to the patch pipette solution was used as previously described (3) to analyze the morphology of the GFP-labeled recorded cells; double-labeled recorded cells were examined using a Leica TCS-SL confocal microscope equipped with Argon and He/Ne laser sources.

RESULTS

Granule cells without any evoked synaptic input or spontaneous activity [belonging to Class 1; see ref. (3)] were considered; RMP of these cells was - 42.9 ± 1.2 mV (mean ± sem), IR was 6500.6 ± 284.4 MΩ and C was 8.6 ± 0.4 pF (n = 118).

Puff application of 100 μM GABA, in the presence of 10 μM CNQX and 50 μM AP5, elicited an inward current in all the analyzed cells (n = 15) at holding potentials of - 80 mV (Fig. 1A). The GABA current showed a peak of 80.1 ± 14.2 pA followed by a plateau whose amplitude was 10.4 ± 1.6% of that of peak, suggesting a GABA receptor desensitization (Fig. 1A). This response was significantly reduced by 10 μM bicuculline methiodide (BMI) application (tested in n = 5) (peak to 37.4 ± 7.0% and plateau to 46.7 ± 7.8%, respectively; Student's t-

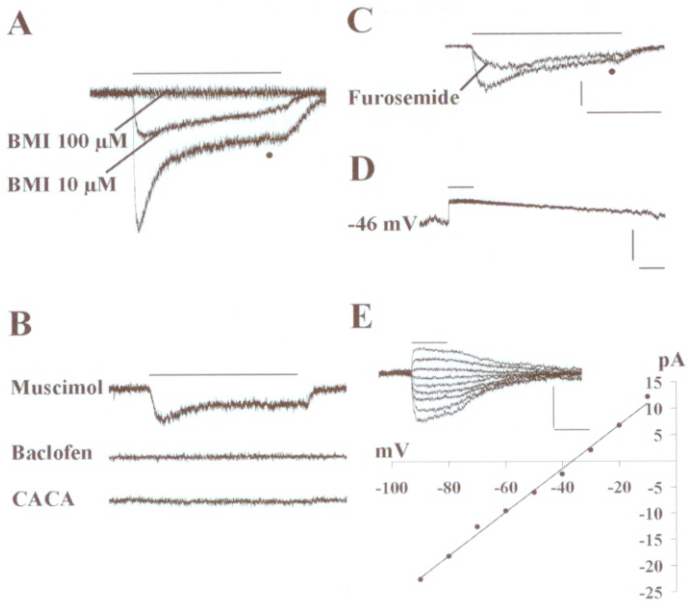

Fig. 1. *A. Response evoked by puff application of GABA (100 µM). GABA-evoked current was partially blocked by 10 µM BMI, and totally suppressed by 100 µM BMI; B. Response evoked by muscimol puff application. Baclofen and CACA puff application did not evoke any current. C. Effect of furosemide on muscimol-evoked response. A, B and C: horizontal bar: 7 s; vertical bar: 20 pA. D. Patch-perforated recordings. I/V curve of current evoked by muscimol puff application. Inset illustrates currents recorded at the indicated membrane potentials. Horizontal bar: 2 s; vertical bar: 20 pA. E. Depolarization induced by puff application of muscimol. Horizontal bar: 2 s; vertical bar: 20 mV. The bar at the top of the drawings shows duration of drug application. The dot below the trace marks the point in time when the amplitude of plateau currents was measured.*

test: $p < 0.005$ in both cases) and totally eliminated by 100 µM BMI (Fig. 1A), suggesting that GABA-evoked currents in these non contacted immature neurons were mediated by $GABA_A$ receptor types, and that these receptors displayed a differential sensitivity to BMI. To directly investigate the possible expression of different types of functional GABA receptors, the responsiveness of the analyzed cells (n = 25) to $GABA_A$, $GABA_B$ and $GABA_C$ receptor agonists was tested. All recorded cells responded to puff application of 30 µM muscimol ($GABA_A$ receptor agonist) showing an inward current at a holding potential of -80 mV with a shape similar to that elicited by 100 µM GABA application (Fig. 1B) and a reversal potential of - 68.3 ± 2.5 mV (calculated in n = 6); once again, this response was totally suppressed in the presence of 100 µM BMI. Puff application of 10 µM baclofen and 100 µM CACA ($GABA_B$ and $GABA_C$ receptor agonists, respectively) did not evoke any current in any of the analyzed cells (Fig. 1B), confirming that GABA currents in these immature synaptically-silent neurons were exclusively mediated by $GABA_A$ receptors.

To further characterize the pharmacology of this $GABA_A$ receptor-mediated current, the modulator furosemide, a compound acting as a selective, non-competitive antagonist of α4- or α6-containing $GABA_A$ receptors (21), was used. While the $GABA_A$ receptor α6 subunit is expressed in cerebellum (32), the α4 subunit is expressed in developing DG and has been detected extrasynaptically in mature granule neurons of adult DG. Receptors assembling

these subunits show a high affinity for GABA and do not desensitize on the prolonged presence of agonist (24, 33). A 300 μM furosemide application (n = 7) significantly reduced the $GABA_A$ receptor-mediated current elicited by 30 μM muscimol (peak to 67.6 ± 5.3% and plateau to 70.0 ± 6.9%, respectively; Student's t-test: $p < 0.005$ in both cases), suggesting the presence of functional $GABA_A$ receptors containing α4 subunit (Fig. 1C).

The gramicidin-perforated patch-clamp technique, which maintains intracellular chloride concentration intact (13), was used to accurately assess whether the effect of $GABA_A$ receptor activation was depolarizing, as it is in developing brain. These experiments were carried out in current-clamp mode without modifying cell resting membrane potential (which was - 43.2 ± 1.7 mV). In all the recorded cells (n = 10) the $GABA_A$ receptor-mediated current, elicited by a 30 μM muscimol application, had a depolarizing effect (Fig. 1D). The reversal potential of $GABA_A$ receptor current, recorded in voltage-clamp mode, was - 34.7 ± 3.3 mV (evaluated in n = 4) (Fig. 1E); this potential can be considered the E_{Cl} because the reversal potential of $GABA_A$ receptor current measured using whole-

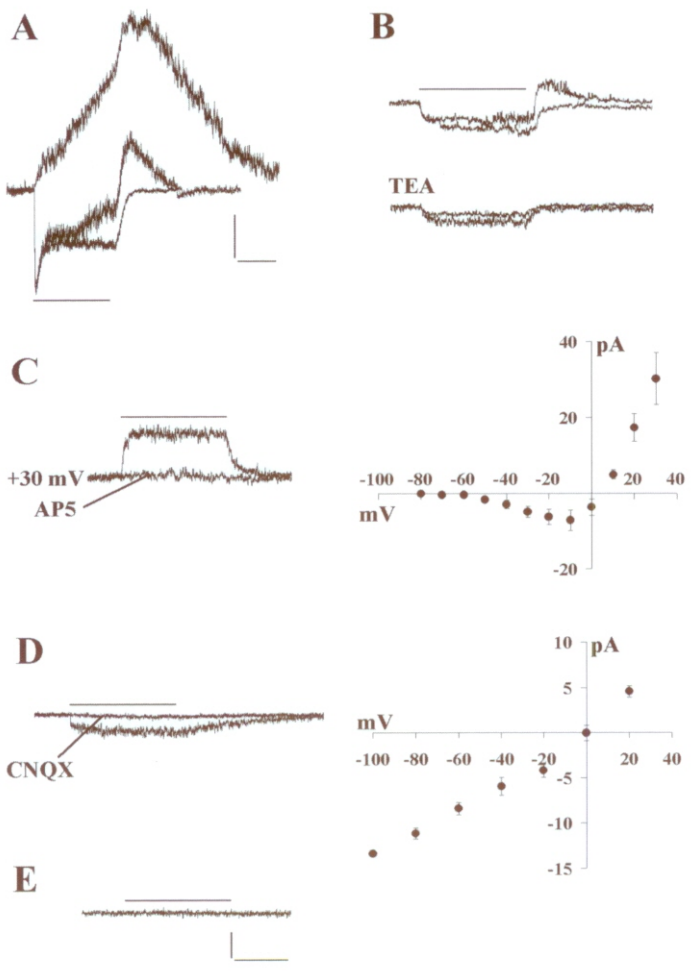

Fig. 2. - *A. Response evoked by puff application of 100 μM glutamate recorded at - 40 mV, - 20 mV and 0 mV. An outward current appears at - 20 mV continuing after drug application. Horizontal bar: 7 s; vertical bar: 50 pA. The bar at the bottom of the drawing shows duration of drug application. B. Current evoked by 300 μM NMDA puff application at - 40 mV and - 20 mV. An outward current is visible at - 20 mV after the drug application; this current is suppressed by 2 mM TEA. C. NMDA receptor-mediated current is fully blocked by 50 μM AP5; I/V curve shows typical features of NMDA receptor-mediated current. D. Current elicited by 100 μM Kainate puff application, blocked by 30 μM CNQX, and relative I/V curve. E. No current was evoked by 100 μM ACPD puff application. B, C, D, and E: horizontal bar: 7 s; vertical bar: 20 pA. The bar at the top of the drawings shows duration of drug application.*

cell configuration recordings (see above) was similar to that resulting from Nerst's equation (- 70.5 mV). Thus, the calculated intracellular chloride concentration was about 34 mM.

Puff application of glutamate elicited an inward current at a holding potential of - 80 mV (n = 3), which showed a complex shape at depolarizing potentials (about - 20 mV), due to the appearance of an outward current continuing after the application (Fig. 2A). Puff application of 300 μM NMDA, in the presence of 10 μM glycine and 20 μM BMI, evoked an inward current at a holding potential of - 40 mV (n = 4) (Fig. 2B); a further depolarization of cell membrane induced an outward current, similar to that evoked by the glutamate puff application, that was totally blocked by 2 mM TEA (Fig. 2B). The voltage-dependence, high sensibility to TEA and kinetics of this outward current suggested that it was mediated by BK type Ca^{2+}-activated K^+ channels (44). Puff application of 300 μM NMDA, in the presence of 2 mM TEA in addition to the other drugs, elicited a current in 14 of 16 recorded Class 1 cells, showing a I/V curve typical of NMDA receptor-mediated current; the current was completely suppressed by a 50 μM AP5 application (Fig. 2C). 20 of 26 recorded cells responded to a puff application of 100 μM kainate in the presence of 100 μM cyclothiazide and 20 μM BMI; the current showed a I/V curve typical of AMPA/Kainate receptor-mediated current (Fig. 2D). No current was evoked by the glutamate metabotropic receptor agonist ACPD (100 μM) puff application (n = 12) (Fig. 2E).

Seven days after retroviral infusion, a number of GFP-positive cells were visible in the hilar border of the GCL. All the recorded labeled cells (Fig. 3) showed morphological features typical of Class 1 cells (3). The RMP of these GFP-positive cells was - 45.1 ± 2.5 mV, the IR was 7005.7 ± 1010.5 MΩ, and C was 8.6 ± 0.8 pF (n = 13). These newly-generated neurons did not display synaptic activity evoked by perforant pathway or by granule cell layer stimulation, and did not showed spontaneous synaptic activity. All the recorded labeled cells exhibited voltage-gated K^+ channels, and most of them also showed voltage-gated Na^+ channels giving rise to a rudimentary spike; no currents mediated by voltage-gated Ca^{2+} channels were recordable. GFP-positive cells, showing these electrophysiological features, responded to puff application of GABA and glutamate (Fig. 3). GFP-positive cells exhibiting morphological and electrophysiological features typical of radial glia were also observed, but they were not considered.

DISCUSSION

The present results show that in adult rat hippocampal DG synaptically-silent immature granule neurons [belonging to Class 1 following previous classification; see ref. (3)] are all responsive to GABA in slice and most of them are able to respond to glutamate, indicating that in adult rat brain as well, immature neurons do express functional GABA and glutamate receptors before they are reached by functional synapses, as occurs in ontogenesis. Moreover, we found that 7-day-old adult-generated neurons exhibit the same electrophysiological and morphological features of Class 1 cells and show GABA and glutamate functional receptors in their membrane, as well; these results point out that most Class 1 cells are in their first week of life when the period of maximal newborn cell death occurs and a hippocampus-dependent learning can rescue many of them.

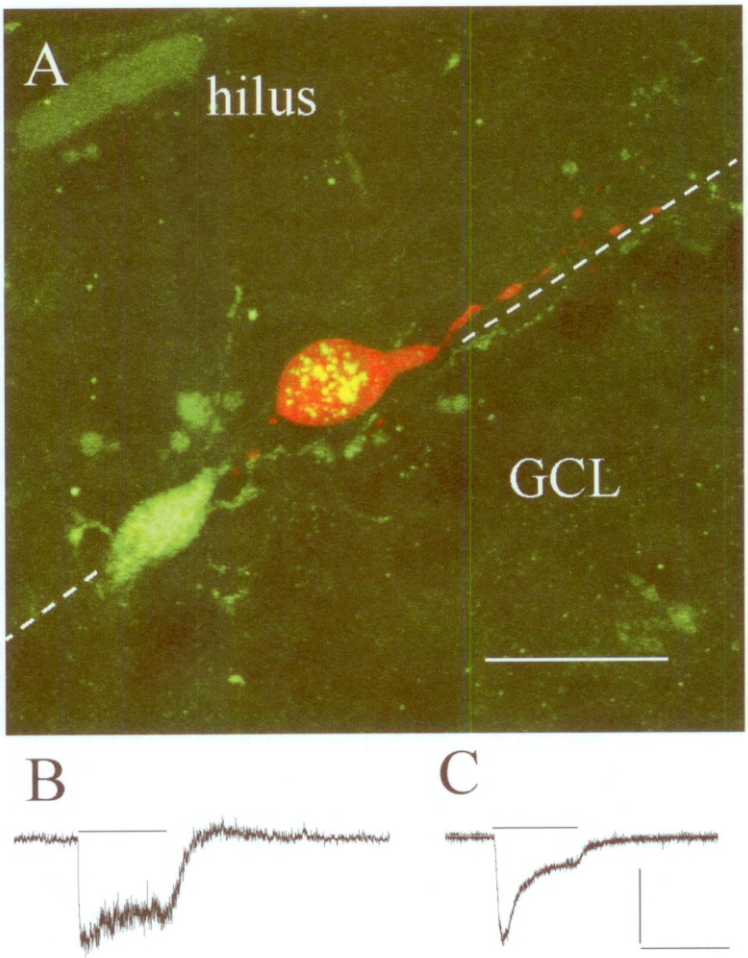

Fig. 3. - *A. Laser scanning confocal image of a cell double-labeled for GFP (green) and biocytin (red), located at the hilar border of the granule cell layer (GCL), marked by a dashed line. In the picture, another single-labeled GFP-positive cell not recorded is visible. Bar = 10 μm. B. Response of 7-day-old GFP-positive cell evoked by puff application of 100 μM glutamate at - 10 mV. C. Current evoked in 7-day-old GFP-positive cell by 100 μM GABA puff application at - 80 mV. Horizontal bar: 14 s; vertical bar: 50 pA. The bar at the top of the drawings shows duration of drug application.*

Our findings obtained in rat GCL are in agreement with that found recently by Esposito *et al.* (14) in female mice undergoing spontaneous motor activity, a condition known to increase DG precursor proliferation (42), to enhance hippocampal levels of neurotrophic factors, such as BDNF (23), that in turn could affect neurogenesis DG (38), and to change dendritic structure and complexity in adult DG granule cells (37). Therefore, species, sex and different experimental conditions do not seem to result in differences in the expression of functional neurotransmitter receptors before synaptogenesis. Our results are also consistent with the findings of Wang *et al.* (46) in a heterogeneous cell population of adult transgenic mouse GCL, and different from those of Tozuka *et al.* (40) that in the same tran-

sgenic mice did not observe any response to either AMPA or NMDA applications in newly-generated cells with only GABAergic synaptic input. Taken together, comparisons between data obtained in different rodent species and diverse experimental conditions show that synaptically-silent immature neurons of DG display a fundamental homogeneity in their capability to respond to GABA, while some differences in the time of emergence of functional glutamatergic receptors are reported.

The present data show that glutamate and GABA receptor-mediated currents elicited in immature, synaptically-silent neurons of adult rat GCL are caused by ionotropic receptor activation. None of these cells responded to metabotropic glutamate or GABA receptor agonists. However, since metabotropic receptor signaling occurs via G protein activation and its effects do not necessarily involve activation of membrane currents, the presence of glutamate and GABA metabotropic receptors cannot be ruled out.

GABA responses observed in non-contacted immature neurons were relatively resistant to bicuculline and significantly reduced by the allosteric modulator furosemide, thus exhibiting a pharmacological profile similar to that of granule cells in developing DG (24, 26). In embryonic and neonatal brain, such pharmacological characteristics are thought to be correlated with the pre-eminent expression of specific, developmentally-regulated $GABA_A$ receptor subunits (namely $\alpha3$, $\alpha4$, $\gamma2$) and are associated with physiological properties typical of immature GABA receptors, i.e. high affinity for ligand and a relative lack of desensitization, which would increase the likelihood of paracrine receptor activation by low levels of extracellular ligand (12, 36). Paracrine activation of $GABA_A$ receptors also occurs in adult DG, where high-affinity, extrasynaptic $GABA_A$ receptors enriched in $\alpha4$ subunits of mature neurons can be activated by spilled-out GABA thereby mediating a tonic form of inhibition (33). All of these findings suggest that functional $GABA_A$ receptors expressed by synaptically-silent immature granule cells in adult DG have a subunit composition that make them suitable to "sense" low levels of extracellular GABA. Granule cell somata are densely targeted by GABA-releasing axon terminals mainly originating from hilar inhibitory interneurons (28), and it is conceivable that spilled-out GABA may reach nearby developing cells and paracrinely activate their high-affinity GABA receptors. This hypothesis is supported by recent evidence showing that GABA-mediated paracrine signaling indeed occurs in GCL immature neurons in adult transgenic mice through the activation of $\alpha2/\alpha4/\alpha5$ subunits-enriched $GABA_A$ receptors (34).

GABA exerts depolarizing, excitatory effects in early developing brain (27, 35), presumably due to high $[Cl^-]_i$ maintained by unopposed inward Cl^- transport (7, 36). Using gramicidin-perforated-patch recordings, which leave genuine $[Cl^-]_i$ undisturbed (13), we found that in Class 1 granule cells $[Cl^-]_i$ was remarkably high (around 34 mM) and E_{Cl} significantly more depolarized than resting membrane potential. These findings clearly show that in adult rat brain as well, the activation of $GABA_A$ receptors in immature, non-contacted neurons invariably produces membrane depolarization, and are in line with recent data obtained in transgenic mice (34, 40) showing GABA-evoked depolarizing effect in immature DG neurons that receive only GABAergic synaptic inputs, and in normal mice showing the same depolarizing effect in synaptically-silent immature neurons (16). This depolarizing effect of GABA-evoked current has been recently shown to be essential for synapse formation and dendritic development in adult mice DG (16).

Membrane depolarization is thought to play a crucial role in triggering glutamate- and GABA-mediated trophic actions in the early phases of brain development by allowing transient increases of $[Ca^{2+}]_i$ via activation of voltage-gated Ca^{2+} channels (VGCCs) or by removing magnesium block on NMDA receptor channels (6, 7, 36). In this investigation, we have shown that all Class 1 cells express $GABA_A$ receptors and the vast majority of them exhibit AMPA and NMDA receptor-mediated responses, showing that most non-contacted immature granule cells co-express functional ionotropic receptors for both GABA and glutamate. In particular, the co-expression of high-affinity, depolarizing $GABA_A$ receptors and Ca^{2+}-permeant NMDA receptors in highly immature neurons, such as Class 1 cells, lacking functional VGCCs (3), may have interesting functional implications. We have shown that GABA-evoked depolarization can shift the membrane potential of Class 1 cells to values at which the block of NMDA-mediated currents by magnesium is partially removed (see Fig 1D, 2C). It is thus tempting to speculate that in early differentiating cells, GABA signaling may interact with NMDA receptor activation, thereby allowing an inflow of Ca^{2+} which may trigger molecular changes that eventually improve cell survival (41). Data showing that in adult GCL endogenous ligands for NMDA receptors can be released from several possible sources, strengthens this hypothesis: in addition to axon terminals originating from excitatory interneurons and directly contacting granule cells (28), it is now well-established that also astrocytes can release classical neurotransmitters, such as glutamate and GABA (45). Moreover, recent data showing that aspartate is co-released with GABA from nerve endings assumed to be inhibitory and contacting DG granule cells (19), point to an additional common source of endogenous release of GABA and NMDA receptor ligands.

Taking all this into account, one can speculate that the co-expression of functional $GABA_A$ receptors, most of which contain the $\alpha 4$ subunit that gives these receptors a high affinity for GABA and relative lack desensitization, and ionotropic glutamate receptors might allow 7-day-old, synaptically-silent immature neurons of adult rat GCL to be affected by extracellular neurotransmitter levels that change during ongoing hippocampal circuitry activity; this could help explain learning-induced survival of newly-generated, synaptically-silent neurons. This hypothesis can be supported by the most recent findings by Ge *et al.* (16) showing in mice that newly-generated granule maturation can be affected by neuronal network activity through tonic $GABA_A$ receptor activation.

SUMMARY

The fate of adult-generated neurons in dentate gyrus is mainly determined early, before they receive synapses. In developing brain, classical neurotransmitters such as GABA and glutamate exert trophic effects before synaptogenesis. In order for this to occur in adult brain as well, immature non-contacted cells must express functional receptors to GABA and glutamate. In this investigation, patch-clamp recordings were used in adult rat dentate gyrus slices to assess the presence and analyze the characteristics of GABA- and glutamate-evoked currents in highly immature, synaptically-silent granule cells. Whole-cell patch-clamp recordings showed that all the analyzed cells responded to puff application of GABA and most of them responded to glutamate. Currents evoked by GABA were mediated

exclusively by $GABA_A$ receptors and those elicited by glutamate were mediated by NMDA and AMPA/Kainate receptors. $GABA_A$ receptor-mediated currents were reduced by furosemide, which suggests that synaptically-silent immature neurons express high-affinity, α4-subunit-containing $GABA_A$ receptors. Gramicidin-perforated-patch recordings showed that $GABA_A$ receptor-mediated currents exerted a depolarizing effect due to high intracellular chloride concentration. Synaptically-silent immature cells shared morphological and electrophysiological properties with GFP-expressing, 7-day-old adult-generated granule layer cells, indicating that they could be in the first week of life, the period of maximal newborn cell death. Moreover, the presence of functional GABA and glutamate receptors was confirmed in these GFP-expressing cells. Present findings are mostly consistent with previous data obtained in female mice undergoing spontaneous activity and in transgenic mice, except for some inconsistencies about the presence of functional glutamatergic receptors. We speculate that adult-generated, non-contacted granule cells may be able to sense activity-related variations of GABA and glutamate extracellular levels. This condition is necessary, even if not sufficient, for these neurotransmitters to have a direct role in addressing cell survival.

Acknowledgments: - We wish to thank Dr Andrea Frontini, Istituto di Morfologia Umana Normale, Università Politecnica delle Marche (Ancona, Italy), for his precious technical assistance with the confocal microscope.

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