

Heterogenous Properties of Dentate Granule Neurons in the Adult Rat

Sabrina Wang,¹ Brian W. Scott,² J. Martin Wojtowicz²

¹ Kinsmen Lab, University of British Columbia, 2255 Wesbrook Mall, Vancouver, British Columbia V6T 1Z3, Canada

² Department of Physiology, Medical Sciences Building, University of Toronto, Toronto, Ontario M5S 1A8, Canada

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ABSTRACT: Postnatal neurogenesis contributes substantially to the neuronal population of the adult dentate gyrus. We report here that the neurons located in the deep aspects of the granule cell layer, near the proliferative zone, have different properties from those located in the superficial layers. The former group of neurons, tentatively designated as young, can be readily identified in a standard hippocampal slice preparation by morphological, immunohistochemical, and electrophysiological criteria. Electrophysiological recordings and imaging with Lucifer yellow from these neurons in the standard hippocampal slice preparation showed one or two main dendrites and conically shaped branches possessing varicose protrusions. These features are in agreement with the appearance of the same population of young neurons immunopositive for TOAD-64, a

marker for immature neurons. In disinhibited slices, these putative young neurons are distinguished from the mature neurons, located in the superficial granule cell layer, by showing paired pulse facilitation and having a lower threshold for induction of long-term potentiation. The putative young neurons are completely unaffected by GABA_A inhibition and always display robust long-term potentiation. In contrast, the mature neurons never produce long-term potentiation when the GABA_A inhibition is intact. We propose that the heterogeneity of the functional properties of the granule neurons is related to the ongoing neurogenesis in the adult animals. © 2000

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In the hippocampal dentate gyrus, new neurons are continuously produced in adult mammals. These new neurons are generated from a local population of progenitor cells located in the subgranular zone (Gage et al., 1998; Cameron et al., 1993). The rate of this process slows down with advanced age (Kuhn et al., 1996) or during stress (McEwen et al., 1994) but can be accelerated by various traumatic events such as seizures (Parent et al., 1997) or ischemia (Liu et al., 1998). Postnatal neurogenesis follows the prenatal developmental pattern (Cowan et al., 1980), so an

outside-in gradient of cell ages is established with the oldest cells located on the outside (molecular) and youngest ones on the inside (hilar) edge of the granule cell layer (Crespo et al., 1986; Altman and Bayer, 1990; Seki and Arai, 1993; Kuhn et al., 1996).

It has been shown that these new adult-generated neurons develop dendritic and axonal processes and receive synaptic contacts on their cell bodies (Stanfield and Trice, 1988; Markakis and Gage, 1999). Neurons colabeled for the mitotic indicator bromodeoxyuridine (BrdU) and markers of early developmental stages, PSA-NCAM and TOAD-64 (Seki and Arai, 1993; Scott et al., 1998; Gould et al., 1999), are all found at the inner edge of the granule cell layer and are characterized by one or two main dendrites and

Correspondence to: J. M. Wojtowicz
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compact dendritic branches. In young adult rats, many such neurons are present since they are rapidly produced in the first few months, with the average number of new cell production estimated at about 1000/day (Bayer, 1982; Markakis and Gage, 1999).

Recent studies have shown that changes in the rate of neurogenesis may be influenced by environmental factors such as complex environment (Kempermann et al., 1997) or hippocampal-dependent learning (Gould et al., 1999). Since changes in the relative proportion of young cells may influence hippocampal functioning, it is important to establish whether the properties of the new neurons are in any way different from those of the old ones. Electrophysiological recordings from individual granule neurons in hippocampal slices have revealed many forms of synaptic plasticity which include short-term and long-term facilitation and depression. The synaptic properties of neurons vary widely with the age of animals (Ben-Ari et al., 1997; Barnes, 1994). Properties of granule neurons in the dentate gyrus, especially at the early developmental stages, have been investigated in detail (Liu et al., 1996; Trommer et al., 1995). However, these studies are potentially complicated by a mix of cellular ages. During the first month after birth, many of the granule neurons are newly born, some are more mature, and others could be in the process of regression which is believed to be prominent during the early development (Schlessinger et al., 1975). Thus, one may encounter cells of widely differing ages and possibly with different properties.

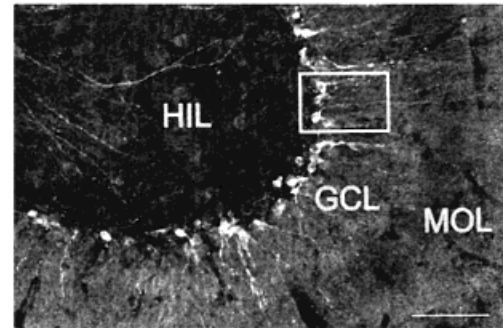
In this study, we undertook a novel approach by recording from hippocampal slices taken from young adult animals (1–2 months) and comparing the properties of the putative adult-generated neurons located in the deep granular layer to those on the outside of the granular layer which are chronologically oldest. We identified two morphologically and physiologically distinct populations of neurons which could play different roles in hippocampal-dependent behaviors.

MATERIALS AND METHODS

Animals and Electrophysiology

Six- to nine-week-old male Wistar rats (Charles River) were used in these experiments. Animals were cared for according to the protocols approved by the University of Toronto Animal Care Committee. Hippocampal slices were prepared with standard methods described in our previous publications (Wang et al., 1996; Wang and Wojtowicz, 1997). In short, animals were anesthetized with halothane and decapitated, their brains were removed and hippocampi were dissected. Four hundred-micrometer-thick slices were

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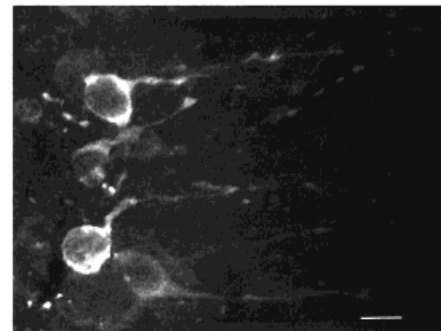


Figure 1 Localization and morphology of young neurons in the adult dentate gyrus of the hippocampus. (a) Transverse histological section of the dentate gyrus shows TOAD-64-positive neurons at the inner edge of granule cell layer (GCL) near the hilus (HIL). TOAD-64 is a specific postmitotic marker labeling only young neurons. Some of the axons traversing the hilus, presumably mossy fiber projections from the young neurons, are also labeled. Mature neurons are found throughout the GCL, but the oldest ones are restricted to the outer edge of GCL near the molecular layer (MOL). Calibration bar = 100 μm . (b) The region boxed in (a) is shown at higher magnification. Typically, the young neurons had one primary dendrite emerging from the cell body and varicose dendrites. Calibration bar = 10 μm .

cut with a tissue chopper and placed in a holding chamber for at least 1 h. For recordings, the slices were placed in a chamber continuously perfused with artificial cerebrospinal fluid at 30–32°C. The whole-cell recordings were done by advancing the recording pipette towards the inner 20- μm band or the outer 20- μm band of the granule cell layer in the dentate gyrus (Figs. 1–3). This approach proved to be effective in locating the young and the old neurons, respectively. In all experiments, the location of recorded neurons was confirmed in fixed slices. Data from neurons outside these two locations, i.e., in the middle of the granular layer,

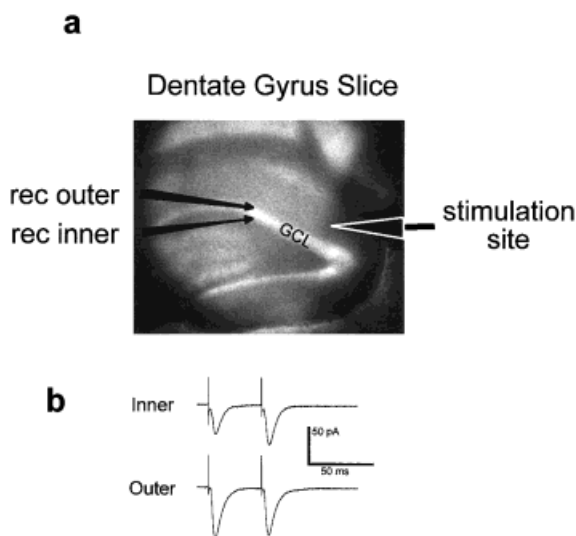


Figure 2 (a) Photograph of a living hippocampal slice viewed through a microscope. The translucent GCL with the typical position for the outer-edge (rec outer) and inner-edge (rec inner) recording electrodes. The stimulating electrode was placed in the middle molecular layer, allowing activation of the afferent medial perforant pathway. (b) Sample traces of evoked excitatory postsynaptic currents (EPSC) from inner and outer neurons. Recordings were done in presence of $10 \mu\text{M}$ BIC to block IPSCs. The inner neuron shows paired-pulse facilitation. The outer neuron shows slight paired-pulse depression.

have not been included in this report. We included 1 mg/mL Lucifer yellow (potassium salt; Molecular Probes) in the internal solution for imaging cell morphology. The other components of the solution included (in mM): 142.5 potassium gluconate, 17.5 KMeSO_4 , 8 NaCl, 10 HEPES, 0.1 EGTA, 2 MgATP, 0.2 GTP, pH 7.3, osmolality 290–300 mOsm. In experiments involving a pairing protocol for induction of long-term potentiation (see Fig. 7), we used a modified intracellular solution with the following ingredients (in mM): 142 Cs gluconate, 17.5 Cs chloride, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.2 GTP, 10 QX-314. The pH was adjusted with CsOH to 7.3 and the osmolality was adjusted to 300 mOsm. This solution was used to enable low-noise recordings at depolarized holding voltages. We used 1 mg/mL Lucifer yellow (lithium salt; Molecular Probes) for imaging. The integrity and stability of the recordings were determined by monitoring the series resistance (20–30 $\text{M}\Omega$) and the holding current.

Synaptic responses were evoked by electrical stimulation via tungsten electrodes in the middle molecular layer (medial perforant pathway) of the suprapyramidal blade (Fig. 2). The frequency of stimulation was 0.2 Hz for baseline responses. Synaptic responses were recorded in the voltage clamp mode with a holding membrane potential at -80 mV . This was very near the initial resting potential obtained in these neurons immediately after breakthrough and generally required only a few pA of holding current. In

most experiments, the stimulation intensity was regulated to obtain excitatory postsynaptic currents (EPSC) of approximately 50 pA in amplitude. This required stimulating currents of 0.1–0.4 mA in amplitude and 0.02 ms in duration. Long-term potentiation (LTP) was induced with four trains at 100 Hz, each lasting 500 ms at baseline intensity and repeated every 5 s. The trains were paired with postsynaptic depolarization to -20 mV . This protocol was found to be the most effective among several others tested in a previous study (Wang et al., 1996). In experiments described in Figure 7, LTP was induced by pairing a weak 2-Hz afferent stimulation, producing evoked currents in the 5- to 10-pA range, with postsynaptic depolarization under voltage clamp. The exact extent of depolarization at the remote dendritic location where synapses are located is not known but was estimated to be slightly larger for the mature neurons on the basis of less positive reversal potential for AMPA-dependent EPSCs. With $10 \mu\text{M}$ bicuculline (BIC) and $50 \mu\text{M}$ D-APV present, to block γ -aminobutyric acid (GABA) and *N*-methyl-D-aspartate (NMDA) responses, respectively, the extrapolated reversal for the AMPA component was $+20 \text{ mV}$ in five mature and $+30 \text{ mV}$ in three putative young neurons. There was no significant loss of LTP owing to possible washout of intracellular compounds for at least 2 h. In most cases, cells were tetanized for the first time 10–20 min after breakthrough. In some experiments, $10 \mu\text{M}$ bicuculline methiodide was added to the perfusate to block GABA_A -dependent inhibitory responses.

Confocal Imaging and Immunohistochemistry

After each electrophysiological experiment, slices were fixed overnight in buffered 4% formaldehyde. This was followed by dehydration with alcohol and clearing with methyl salicylate. The Lucifer yellow-filled cells were viewed under a BioRad 600 confocal microscope using $\times 20$ or $\times 40$ objective. Successive optical sections of the dendritic tree were taken by moving the motorized stage of the microscope in the Z axis. In most cases, 15–30 sections separated by 2- to $5\text{-}\mu\text{m}$ steps were sufficient to view the complete dendrite. Composite images were obtained by stacking the optical sections into a two-dimensional image using Co MOS software. In many cases, the mossy fiber axons of the cells, including the synaptic boutons in CA3, were also reconstructed to verify that the cells were granule neurons and not interneurons (not shown). The interneurons were rarely encountered.

The immunohistochemical procedure to show the turned-on after division 64-kD protein (TOAD-64), a marker for immature neurons, was as described previously (Parent et al., 1997; Scott et al., 1998). The TOAD-64 antibodies were generously donated by Dr. S. Hockfield, Yale University, New Haven, Connecticut.

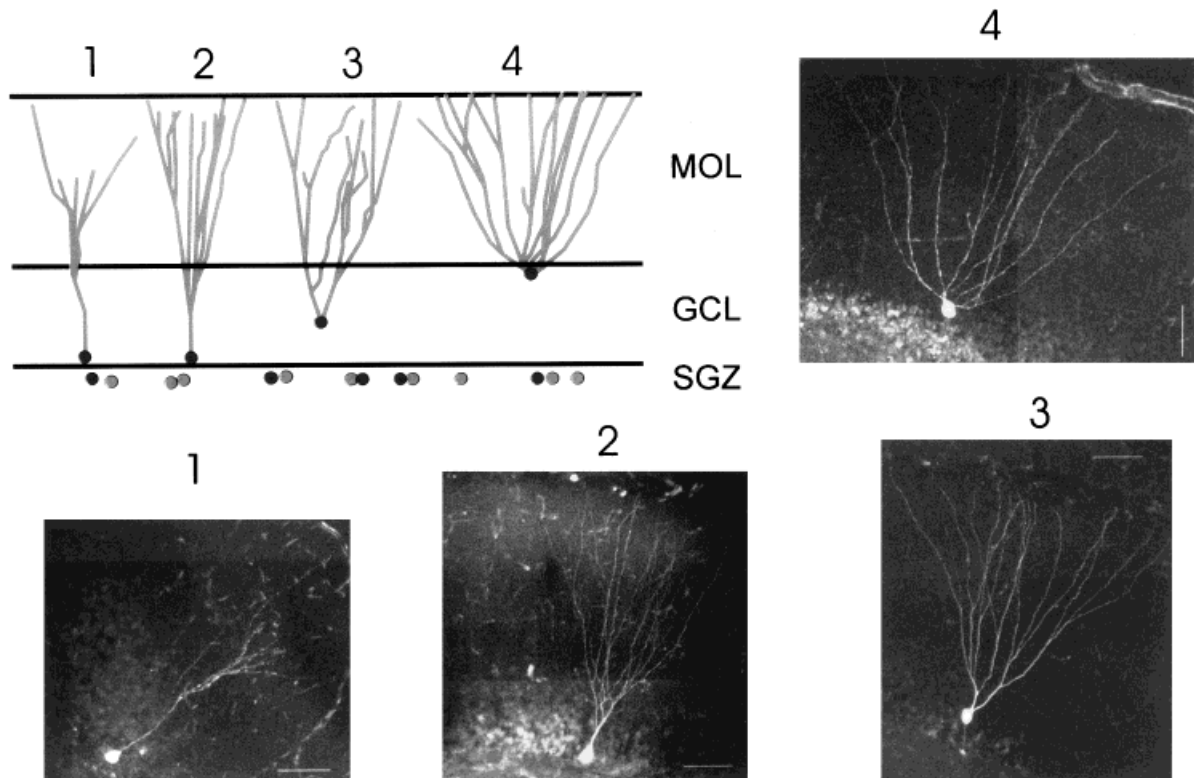


Figure 3 Examples of dentate gyrus granule neurons presumed to be at different developmental stages. Cells were filled with the fluorescent dye Lucifer yellow during whole-cell patch recording in hippocampal slices during the course of this study. The differences in dendritic morphology between putative young neurons (cells 1 and 2) and mature neurons (cells 3 and 4) include the number of primary dendrites, and the width of the dendritic tree (see text). Inset shows schematic summary of the various dendritic forms found in different regions of GCL. The radial pattern of neuronal migration during development results in the gradient of neuronal ages, with cell 1 being the youngest and cell 4 the most mature. Circles in the subgranular zone SGZ represent neuronal precursor cells. Scale bars = 50 μm .

RESULTS

Characteristics of Neurons in Different Regions of the Granule Cell Layer

Immunohistochemical labeling of the adult dentate gyrus shows neurons immunoreactive for TOAD-64, an early postmitotic marker of neurons (Minturn et al., 1995) demonstrating a strict localization of the newly born neurons at the inner edge of the granule cell layer (GCL) (Fig. 1). Whole-cell patch recordings from granule neurons at the border of the GCL and the hilus (HIL) were compared with those near the outer (near the molecular layer) border of the GCL where the newborn cells are never found. Only the neurons localized within 10–20 μm from the borders were included in this report. In each electrophysiological experiment, we measured the input resistance of the neurons for subsequent correlation with certain morphological characteristics such

as shape and number of dendritic branches. On average, the inner neurons had higher input resistance [248 ± 82 standard deviation (S.D.) $\text{M}\Omega$; $n = 12$] than the neurons located at the outer edge of the GCL (182 ± 69 ; $n = 14$). This difference was statistically significant ($p < .05$, t test) and probably reflects the generally more compact size of the dendritic tree in the neurons located at the inner edge (Winslow et al., 1999). To verify this observation, in the same sample of neurons we counted 1.5 ± 0.5 primary dendrites extending from the cell body in neurons on the inner edge, in contrast to the neurons located on the outer edge, which had 4.1 ± 0.9 primary dendrites. Also, in the same groups of neurons, we detected a statistically significant difference in the magnitude of paired pulse facilitation (PPF) evoked by paired stimuli at short intervals (Fig. 2). For the inner neurons, a 20% facilitation at the interpulse interval of 40 ms was found. In contrast, the outer neurons pro-

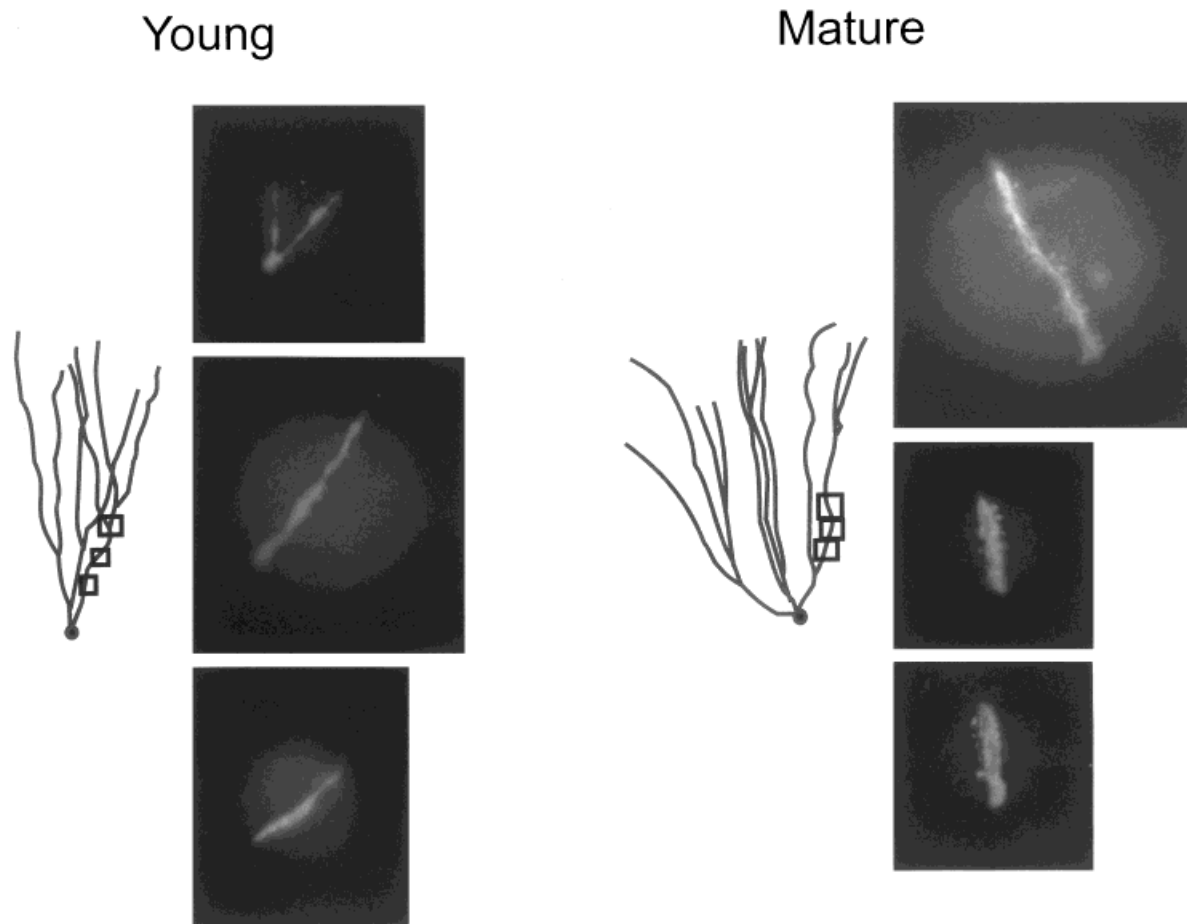


Figure 4 Detailed morphology of dendrites in putative young and mature neurons. A typical thin varicose dendrite in the young neuron, filled with Lucifer yellow, resembles similar processes in the TOAD-64-immunoreactive cells shown in Figure 1. The presumed mature neurons had thicker dendrites with numerous spines. Viewed with a compound microscope, $\times 100$, oil-immersion objective. Inserts show sketches of low-power views of each neuron with the boxes placed on the magnified regions. The locations of the neurons were same as described in Figure 3.

duced no facilitation at the same interval. The difference between the two groups also applies to longer pulse intervals ranging from 40 to 300 ms described in an earlier report (Wojtowicz, 1998). Thus, the differences in the morphological and electrophysiological characteristics and the short-term facilitation between the two groups of neurons provide evidence for functional differentiation among the granule neurons, and suggest that the axonal terminals originating from the same pathway (medial perforant pathway) may give rise to synapses with different facilitating properties, depending on the age of the target neuron.

Representative examples of the neurons encountered in the inner, middle, and outer regions of the GCL are shown in Figure 3. These samples are based on 40–50 neurons in each group. The position and geometry of all neurons studied electro-

physiologically were visualized post-hoc in fixed slices using confocal microscopy and/or compound fluorescence microscope. Although not evident in the two-dimensional reconstructions, the dendritic spread in the z axis of inner neurons was also more compact than that of the outer, presumably mature neurons. It should be stressed that the neurons with the morphological characteristics of the group found on the outer edge of the granule cell layer (cell 4 in Fig. 3) were not found at the inner rim of the granule cell layer, and vice versa, making these morphological criteria very reliable means of categorizing the cells into two groups. The clear gradient of cell shapes does not necessarily imply that in the adult animal neurons of type 1 and 2 migrated and became neurons of group 4, since there is no evidence that the outer neurons become replaced. In

the middle layers (e.g., cell 3 in Fig. 3), a variety of cell types were found with no obvious relation between the position and the morphology.

The dendrites of the inner neurons filled with Lucifer yellow were characterized by varicosities (Fig. 4), similar to those seen on the dendrites of the young neurons identified by TOAD-64, and had only few very thin dendritic spines. This is consistent with the progressive acquisition of dendritic spines by developing granule neurons during the early postnatal period (Cowan et al., 1980). All neurons presented in this report have been identified as granule neurons by their axonal projections through the hilus. The inhibitory interneurons, typically localized just under the GCL, were encountered infrequently and were readily identified by their profuse axonal arborization in the molecular layer (Mott et al., 1997).

Long-term Potentiation (LTP) of Inner and Outer Neurons

Long-term potentiation was studied in 13 inner and 21 outer neurons. The approximate location of the neurons could be determined by viewing living slices under the dissecting microscope, as shown in Figure 2, and directing the recording pipette toward the inner (presumably young) or outer (presumably mature) regions of the GCL. The location was always confirmed by observing the complete Lucifer yellow-filled neurons in fixed slices, as shown in Figure 3. The outer-edge granule neurons did not produce LTP when tetanized in the standard ACSF (0 of 9 neurons) [Fig. 5(a)]. After addition of 10 μM BIC, a GABA_A receptor blocker, the induction of LTP was possible in half (6 of 12) of the neurons. In those neurons which did show significant LTP in the presence of BIC, the magnitude of the enhancement was sometimes very large (up to 350%). A major reason for the complete absence of LTP in these neurons in regular ACSF was a strong GABAergic inhibition, shunting the excitatory responses. This was evident from the enhanced magnitude of evoked EPSCs upon addition of BIC into the perfusate [Fig. 5(a)].

The putative young neurons behaved differently. Regardless of the presence or absence of BIC they produced consistent, robust LTP in 12 of 13 experiments. A typical cell in this group is illustrated in Figure 5(b). The tetanic stimulation produced LTP in normal ACSF solution. Subsequent perfusion of BIC revealed only a slight increase of the EPSC. A summary of all experiments is shown in Figure 6(a). Statistical analysis revealed no significant difference in the magnitude of LTP between cells tested in ACSF or BIC (analysis of variance on ranks, $p > .05$) except

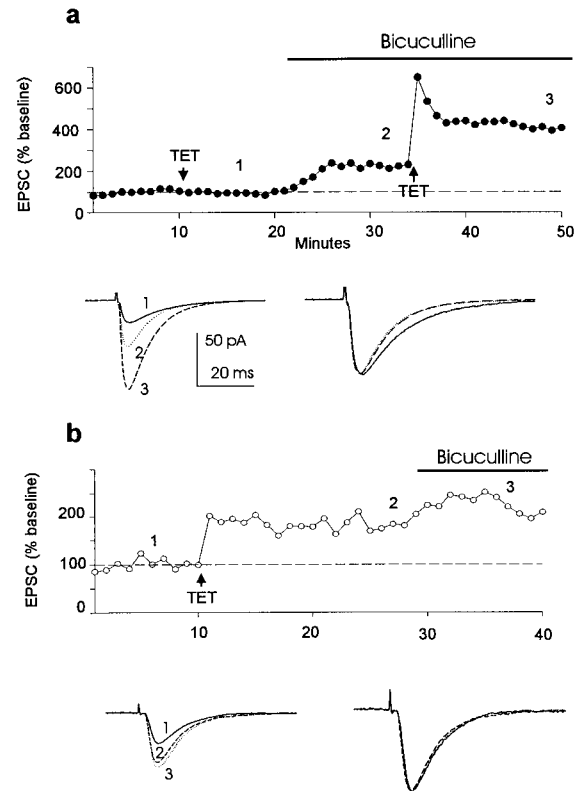


Figure 5 Long term potentiation in granule neurons. (a) Time course of an experiment showing changes in the amplitude of EPSC in a mature granule neuron. Tetanic stimulation (TET) in normal ACSF had no effect. The same type of stimulation in presence of 10 μM bicuculline (BIC) produced robust LTP. Below are traces of EPSCs taken at time points indicated by 1, 2, and 3: 1 = control EPSC in ACSF; 2 = EPSC in BIC; 3 = potentiated EPSC in BIC. The peak of the EPSC was enhanced by BIC, presumably owing to blockade of the GABAergic conductance. Traces on the right have been normalized to the same peak amplitude. The late component of the inward current was attenuated by BIC, consistent with the presence of GABAergic inhibitory postsynaptic current (IPSC) in this neuron. (b) Recording from a putative young neuron showing that LTP can be induced in ACSF without the presence of BIC. This was not observed in mature neurons. Superimposed traces 1, 2, and 3 show that BIC had little effect on the amplitude (left) or time course (right) of the EPSCs. Subsequent tetanization in presence of BIC had no additional effect, suggesting saturation of LTP (not shown).

for the outer edge neurons tested in ACSF. In fact, these neurons demonstrated no significant LTP in ACSF [Fig. 6(a)]. Furthermore, even in the presence of BIC the outer cells produced variable effects and there was no overall statistically significant effect when the data in this group were analyzed (paired t test, $p > .05$). This is apparently due to the extremely large variation in the magnitude of LTP in this group

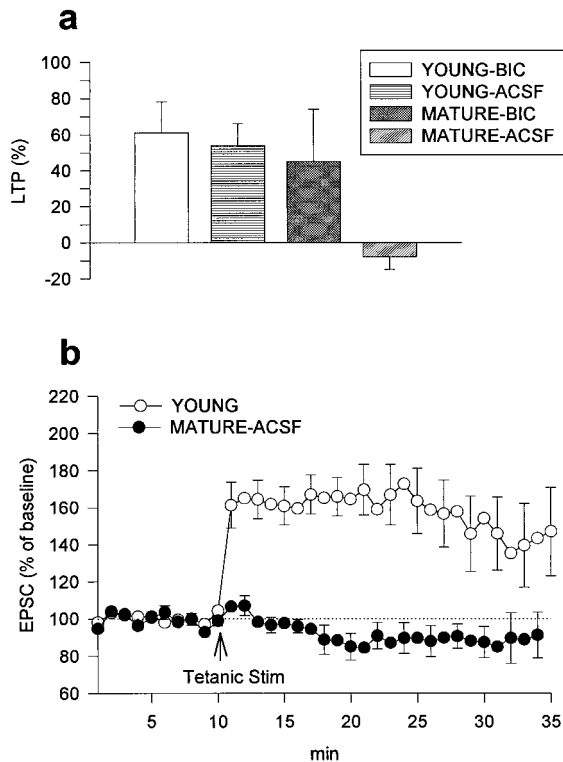


Figure 6 Comparison of LTP between putative young and mature neurons with and without the presence of BIC. (a) Average magnitude of LTP at 20–25 min after induction is shown in the histogram (standard errors indicated). Young-BIC ($n = 8$) and Young-ACSF ($n = 5$) groups were not different from each other, but significantly different from the Mature-ACSF group ($n = 9$) (one-way analysis of variance on ranks with Dunn's pairwise comparison at $p < .05$). The variability in the Mature-BIC group ($n = 12$) was large. Both mature groups lacked statistically significant LTP (paired t test, $p > .05$). (b) Time course of LTP in two subsets of experiments from the four groups described above. The two young groups were combined (open circles; $n = 13$) since they both produced similar LTP. Their LTP was robust for at least 25 min. The Mature-ACSF group (closed circles; $n = 9$) is plotted separately. The average time course of the Mature-BIC group was similar to that of the young group, but had very large error bars, which were omitted for clarity.

(0–350%). The time course of LTP in inner and outer neurons is shown in Figure 6(b). In this figure, we combined the inner ACSF and inner BIC groups, since they were not different from each other, and plotted them against the outer ACSF group, which had no LTP. Figure 6(b) includes average data for an interval of 25 min after the tetanization. In some experiments, we monitored LTP for up to 1 h.

To test whether the relative absence of LTP in the outer group of neurons was solely due to our particular tetanizing paradigm resulting perhaps in insuffi-

cient depolarization of the neurons, we conducted a separate series of experiments with the use of a different induction protocol. In these experiments, we paired the 2-Hz presynaptic stimulation with the postsynaptic depolarization to -20 mV under voltage-clamp control using microelectrodes filled with cesium gluconate to block potassium conductances and QX-314 to block sodium conductance and $10 \mu\text{M}$ BIC in the perfusate. To assess an adequate voltage control, the evoked EPSCs were monitored intermittently at either $+60$ or -80 mV. In these experiments, the inner neurons produced a consistent (five of five), $77 \pm 21\%$ enhancement of the EPSCs at -80 mV (Fig. 7) similar in magnitude to that obtained with the tetanic stimulation protocol. At $+60$ mV, the effect was smaller, as expected, since the EPSC at this depolarized potential included an NMDA component

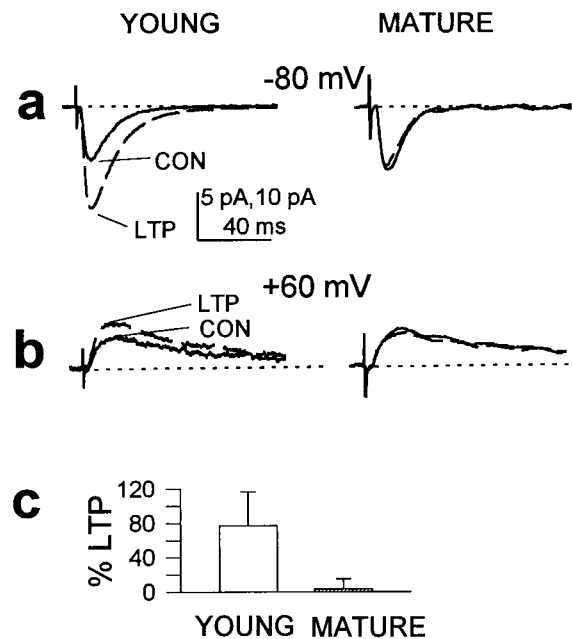


Figure 7 Long-term potentiation (LTP) induced in neurons by pairing of 2-Hz afferent stimulation with postsynaptic depolarization to -20 mV. (a) Averaged EPSCs in a putative young neuron located at the inner edge of GCL before (CON) and 30 min after (LTP) induction of LTP. EPSCs at -80 mV were potentiated by 100%. Traces on the right side were taken from a representative mature neuron located at the outer edge of the GCL which produced no LTP under similar circumstances. (b) Reversed responses at holding potential of $+60$ mV show sufficient voltage control to depolarize neurons substantially above the values used for induction (-20 mV) in both neurons. (c) Comparison of LTP, measured in two groups of neurons at -80 mV ($n = 5$, means and S.E. indicated). Horizontal calibration bar applies to all traces. Vertical bar is 5 pA for young neuron and 10 pA for mature neuron.

which does not potentiate in such experimental conditions (Liao et al., 1995; Isaac et al., 1995). In contrast, the outer neurons did not produce a significant LTP ($3 \pm 12\%$; $n = 6$) under these conditions. The voltage control, as judged by the reversal of evoked EPSCs, was in fact somewhat better in the outer neurons (see Materials and Methods), so the lack of LTP was not due to insufficient postsynaptic depolarization. This induction protocol accentuated the difference in the ability of two groups of neurons to produce LTP.

DISCUSSION

We have shown the presence of two functionally and morphologically distinct populations of neurons within the adult dentate gyrus. The mature neurons, located at the outer edge of GCL, have extensive, tulip-shaped, dendritic arborizations consisting of four or five primary branches projecting toward the hippocampal fissure. These neurons lack the ability to produce short-term facilitation or LTP. The GABA_A inhibition is an important factor, but not the sole one responsible for the lack of LTP. The strong GABA_A inhibition is a characteristic feature of the whole population of granule neurons and is not dictated by the specific experimental conditions employed in this study. For example, it is also seen with less invasive field potential recordings (Hanse and Gustafsson, 1992). The inhibitory tone is also present *in vivo*, although it may be less strong than *in vitro* depending on the experimental conditions (Hanse and Gustafsson, 1992; Nosten-Bertran et al., 1996; Errington et al., 1997). Generally, the magnitude of the LTP seen *in vivo* is 20–30%, which could represent a mix between some neurons which show a large effect and others which do not.

Synaptic responses and LTP in the dentate gyrus are known to be occluded by previous activity (Green and Greenough, 1986; Foster et al., 1996). In agreement with these studies, our experiments showed that the mature neurons as a population, even when disinhibited and artificially depolarized, were not able to produce LTP reliably although in a few neurons LTP was very large [Figs. 5(a) and 6]. All tested neurons appeared to have sufficient NMDA receptors for induction of LTP, and we did not detect an obvious difference in the relative size of the NMDA responses between the two groups (data not shown). The difference between the two groups was even more striking when LTP was induced with weak, low-frequency afferent stimulation instead of the usual high-frequency bursts (Fig. 7), suggesting that the threshold

for LTP induction or expression is raised in mature neurons. One possible explanation for the difference in LTP is the geometry of the dendritic tree, which could generate different local potential changes even with the same synaptic input (Winslow et al., 1999). Verification of this hypothesis will require further modeling and experimental studies.

The putative young neurons are found in the narrow band at the inner edge of GCL and have a conically shaped dendritic tree with one or two primary dendrites. These morphological features have been described previously (Claiborne et al., 1990; Green and Juraska, 1985). The restricted location is an inevitable consequence of the place of birth of these cells in the same region. Their chronological age is not known exactly, although it can be estimated at 1–3 weeks on the basis of double-labeling studies using mitotic indicators and neuronal markers (Seki and Arai, 1993; Cameron et al., 1993; Kuhn et al., 1996). It is also possible that a few of the neurons at the inner edge failed to migrate and are relatively old. However, this group of neurons as a population is certainly younger than the neurons at the outer edge born prenatally or in the early postnatal period (Crespo et al., 1986).

We have shown that the presumed young neurons produce PPF at short interpulse intervals, a feature distinguishing them from the mature granule cell population, which on average show paired pulse depression when stimulated by the medial perforant path afferents (McNaughton, 1980). In fact, our mature group often showed such depression (Fig. 2). Perhaps the most remarkable characteristic of the young neurons is their ability to produce LTP without the need for disinhibition [Fig. 5(b)]. Depolarizing GABA-mediated responses have been shown to excite young neurons, possibly substituting for the AMPA responses (Ben-Ari et al., 1997) at very early developmental stages, but this was apparently not the case here, since the AMPA responses were well developed and bicuculline did not reduce the average magnitude of LTP in this group. Also, the prolonged, polysynaptic GABAergic currents seen in immature granule neurons in neonatal animals (Liu et al., 1996, 1998) were not seen in our study. This discrepancy could represent a difference between the immature neurons in neonatal animals and those in the adults.

Overall, the presumed adult-generated neurons appear to be more plastic and less prone to GABAergic inhibition than the most mature ones. Only a few of the mature neurons produced strong LTP, and all required disinhibition to do so. This lack of LTP cannot be regarded as absolute, since the rate of LTP induction clearly depends on the type of induction

protocol. However, the results do suggest that the threshold for LTP is lower in young neurons. The results are consistent with our hypothesis that the young neurons born into the adult brain are initially unpotentiated and thus naive as far as the synaptic activity is concerned. The LTP-like mechanism may indeed be operating in the intact brain on the young neurons and be responsible for the gradual saturation of the potentiation as the neurons develop. Also, the gradually stronger inhibition may serve to protect the previously potentiated mature neurons from additional activity. The majority of the granule neurons placed in the center of the granule cell layer are likely to have intermediate properties with the characteristics in between the two extreme populations we tested in this study. Further detailed characterization of the young granule neurons and their relative role in a larger population may help us to understand changes in the hippocampal physiology during learning and during pathological states such as stress, epilepsy, and ischemia.

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