Identification of Basolateral Amygdala Projection Cells and Interneurons Using Extracellular Recordings

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Submitted 2 June 2006; accepted in final form 10 July 2006

INTRODUCTION

The role of the basolateral amygdala (BLA) in the formation of appetitive and aversive memories continues to attract much interest (see Baxter and Murray 2002; Everitt et al. 2003; Fanselow and Poulos 2005; LeDoux 2000; McGaugh 2004; Phelps 2006; Walker and Davis 2002). One of the techniques often used to investigate these questions involves extracellular neuronal recordings (reviewed in Holland and Gallagher 2004; Maren and Quirk 2004; Pelletier et al. 2005). However, the BLA contains multiple cell types, including a prevalent group of projection cells and multiple subtypes of local-circuit GABAergic neurons (McDonald 1992), complicating the interpretation of extracellular data. This problem is compounded by the fact that, in contrast with the hippocampus where neurons are segregated in a laminar fashion, different cell types are intermingled in the BLA. As a result, the position of BLA cells cannot be used to infer their identity. Thus it would be of great practical interest to define a set of criteria that could be used to distinguish projection cells from interneurons on the basis of their extracellularly recorded activity.

Intracellular and patch-clamp studies of morphologically identified BLA neurons offer clues as to what properties might be useful to identify BLA cells. Studies performed in vivo (Chen and Lang 2003; Lang and Paré 1997a,b, 1998; Paré et al. 1995) and in vitro (Faber and Sagh 2002, 2003; Faber et al. 2001; Faulkner and Brown 1999; Mahanty and Sah 1998; Martina et al. 2001; Pape et al. 1998; Rainnie et al. 1993; Szinyei et al. 2000; Washburn and Moises 1992) indicate that most principal cells have a comparatively hyperpolarized membrane potential, show little spontaneous firing at rest, and generate trains of long-duration action potentials that exhibit various degrees of frequency adaptation. In contrast, a subtype of BLA interneurons (fast-spiking cells), thought to express parvalbumin and accounting for roughly 50% of BLA interneurons (Mascagni and McDonald 2003), have a comparatively more depolarized membrane potential, are often spontaneously active at rest, and generate trains of short-duration spikes that generally display little or no spike frequency adaptation. The latter property is thought to result from the expression of K+ channels with fast activation kinetics (McDonald and Mascagni 2006).

In addition to their distinct intrinsic membrane properties, synaptic factors also contribute to make interneurons more active than projection cells. Indeed, intracellular studies in vivo (Lang and Paré 1997a,b) revealed that the spontaneous activity of projection cells is dominated by large-amplitude hyperpolarizing potentials generated by a combination of synaptic γ-aminobutyric acid types A and B (GABA and GABA respectively) conductances (Rainnie et al. 1991; Washburn and Moises 1992) and by a synthetically activated Ca2+-dependent K+ current (Chen and Lang 2003; Danboe and Pape 1998; Faber et al. 2005; Lang and Paré 1997b). In contrast, fast-spiking interneurons lack GABA receptors (Huttman et al. 2006; Martina et al. 2001) and their GABA reversal potential is >15 mV positive to that of projection cells, a difference reflecting the presence of cotransporters that accumulate chloride in fast-spiking interneurons and extrude chloride in projection cells (Martina et al. 2001). Together, these contrasting patterns of GABA responsiveness and intrinsic properties should conspire to make projection cells less active than fast-spiking interneurons. Thus differences in firing rates and action potential duration might...
constitute useful criteria to distinguish these two BLA cell types. The present study tested this possibility using extracellular recordings of BLA cells, some of which could be unambiguously identified as projection cells using antidromic activation from known cortical projection sites of the BLA.

METHODS

All procedures were approved by the Institutional Animal Care and Use committee of Rutgers State University, in compliance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services). Adult male cats (2.5–3.5 kg) were preanesthetized with a mixture of ketamine and xylazine [15 and 2 mg/kg, administered intramuscularly (im)] and artificially ventilated with a mixture of ambient air, oxygen, and isoflurane. Atropine (0.05 mg/kg, administered intramuscularly (im)) was administered to prevent secretions. The end-tidal CO2 was kept at 3.7 ± 0.2% and the body temperature was maintained at 37–38°C with a heating pad. The level of anesthesia was assessed by continuously monitoring the electroencephalogram and electrocardiogram.

The bone overlying the regions of interest was removed and the dura mater opened. High-impedance (10- to 12-MΩ) tungsten microelectrodes (FHC, Bowdoin, ME) were lowered to the basolateral nucleus (BL) using a micromanipulator. To physiologically identify BL projection cells, concentric stimulating electrodes were stereotaxically inserted in several cortical projection sites of the cat BL nucleus including the perirhinal cortex, infralimbic cortex, and insula (Krettek and Price 1977a,b; Pare´ et al. 1995; Room and Groenewegen 1986; Smith and Pare´ 1977a,b; Pare´ et al. 1995; Room and Groenewegen 1986). To be classified as antidromic, evoked unit responses had to meet at least two of the following three criteria (Lipski 1981): 1) stable latency (<0.3-ms jitter), 2) collision with orthodromically evoked or spontaneously occurring spikes, and 3) ability to respond faithfully to high-frequency stimuli (300 Hz). The spontaneous and evoked activity of responsive neurons was observed on a digital oscilloscope, digitized at 20 kHz, and stored on disk for off-line analysis. We considered only neurons generating spikes with a high signal-to-noise ratio (≥3).

To determine whether the duration of action potentials constituted a fixed property of each neuron or whether it depended on the exact position of the microelectrode tip with respect to the recorded cell, we performed a “distance test.” In this test, we first located a well-isolated, spontaneously active neuron and then moved the electrode back in 5-μm steps over a distance of ≤110 μm from the recording site where the highest signal-to-noise ratio was observed.

At the end of experiments, the animals were administered an overdose of sodium pentobarbital (50 mg/kg, administered intravenously) and extracellular recording sites were marked with electrolytic lesions (0.6 mA for 10 s). The brains were then taken out, fixed in 2% paraformaldehyde and 1% glutaraldehyde, sectioned on a vibrating microtome (at 100 μm), and stained with neutral red or cresyl violet to reveal electrode placements. The microelectrode tracks were reconstructed by combining the micrometer readings and histology. To be included in the histology, cells had to be histologically confirmed as being located in the BL nucleus.

Analyses were performed off-line with commercial software (IGOR, WaveMetrics, Lake Oswego, OR; Matlab, The MathWorks, Natick, MA) and custom-designed software running on personal computers. Spikes were detected using a window discriminator after digital filtering of the raw waves. All values are expressed as means ± SE.

RESULTS

Spontaneous firing rates of BL neurons

To minimize the risk of missing BL neurons with low spontaneous firing rates, microelectrodes were lowered gradually while applying electrical stimuli to the various stimulation sites (0.3 Hz, 0.1–0.5 mA, 100–300 μs). When an ortho- (Fig. 1A) or antidromically (Fig. 1B) responsive and/or spontaneously active BL neuron with a high signal-to-noise ratio was encountered, a 3-min period of spontaneous activity was recorded. Then, its responsiveness to perirhinal, insular, and infralimbic stimuli was tested. In this case, our goal was to determine whether antidromic discharges could be elicited from one or more of these stimulation sites, to formally establish whether this cell was a projection neuron. It should be stressed that this technique is prone to false negatives because failure to antidromically activate a cell might not mean that the recorded cell is a local-circuit neuron but a projection cell.
whose axon does not end (or course) in close proximity to the stimulating electrodes. Nonetheless, a positive finding, that is the presence of electrically evoked antidromic discharges, can be interpreted as unambiguous evidence that the recorded cell is a projection neuron.

Figure 1B shows an example of a BL neuron that could be positively identified as such because electrical stimulation of the infralimbic (Fig. 1B1) or perirhinal (Fig. 1B2) cortices evoked antidromic spikes characterized by a fixed latency and collision with spontaneously occurring action potentials (Fig. 1B3). Overall, 52.6% of recorded BL neurons (or 41 of 78) were identified as projection cells using this technique. Most of these cells could be antidromically activated from only one of the tested sites (insula, 11.5%; infralimbic, 26.9%; perirhinal, 8.9%), but 5.1% projected to at least two of the tested sites, as in the example of Fig. 1B.

Next, we compared the spontaneous firing rate of physiologically identified projection cells to that of unidentified neurons. We reasoned that because this second group of BL cells should contain a higher proportion of interneurons, differences in the distribution of firing rates between the two groups might allow us to derive criteria that could be used to distinguish projection cells from interneurons. Figure 1C shows the distribution of firing rates in the entire sample (Fig. 1C1), in the subset of physiologically identified projection cells (Fig. 1C2), and in the unidentified neurons (Fig. 1C3).

The spontaneous firing rates of BL cells ranged from 0 to 16 Hz (average 2.1 ± 0.4 Hz; Fig. 1C1). Although nearly 51.2% of projection cells fired ≤1 Hz (Fig. 1C2), we encountered projection cells with spontaneous firing rates as high as 6.8 Hz (Fig. 2). Overall, projection cells had a slightly lower average firing rate (1.3 ± 0.3 Hz, range, 0–6.8 Hz) than that of unidentified neurons (3.1 ± 0.7 Hz; Mann–Whitney U test, P < 0.05). Yet, distributions of firing rates in the two groups overlapped extensively (Fig. 1, C2 and C3), with the exception of a few unidentified neurons (5.1% of entire sample) with unusually high rates of spontaneous firing (9.9–16 Hz). Thus these results suggest that a cutoff firing frequency of 7 Hz might be used to distinguish projection cells from fast-spiking interneurons. However, because counts of parvalbumin immunoreactive cells predict that random samples of BL neurons will include 10% of fast-spiking cells (as estimated in rats; Mascagni and McDonald 2003), and not 5.1% as in our sample, a 7-Hz cutoff is likely to lead to the erroneous classification of nearly half the fast-spiking cells as projection neurons. Moreover, considering that extracellular recordings are biased toward neurons with high firing rates, the latter figure likely underestimates the incidence of misclassifications.

**Spike durations in BL neurons**

Because the above analyses suggest that firing rate is a criterion of limited sensitivity to distinguish projection cells from interneurons, we next turned our attention to spike duration. As reviewed in the Introduction, intracellular and patch-clamp studies of morphologically identified BL neurons indicate that spike duration (typically measured at half-amplitude) is significantly longer in projection cells (1.0–1.5 ms) compared with fast-spiking interneurons (0.5–0.7 ms). However, these recordings are typically obtained from the soma of BL cells. This differs from extracellular recordings where the microelectrode tip (<1 μm) can be positioned at any point around the axonal, somatic, or dendritic compartments of BL cells.

In patch-clamp studies of cortical pyramidal cells, spike durations were shown to vary depending on the cellular com-
part from which the recording was obtained. In particular, dendritic spikes generally last longer (sometimes twice as much) than somatic spikes (e.g., see Gullidge and Stuart 2003; Larkum et al. 2001). In addition, extracellularly recorded axonal spikes were reported to be briefer (0.4–0.7 ms) than somatodendritic spikes in multiple cell types (reviewed in Lemon 1984).

Thus there is reason to suspect that the spikes generated by single cells can have different durations depending on the cellular compartment that is sampled by extracellular microelectrodes, a contention supported by a recent modeling study (Gold et al. 2006). As a result, for interneurons and projection cells to be reliably distinguished on this basis, within-cell variations in spike durations arising from electrode location must be smaller that the variations between cell types. To test whether this is the case in the BL nucleus, we thus performed experiments designed to estimate these two variables.

To assess the impact of microelectrode location on spike duration, we performed a “distance test.” In this test, spikes generated by spontaneously active BL cells (n = 11) were recorded as the electrode was moved in 5-µm steps over a distance of ≤110 µm from the recording site where the highest signal-to-noise ratio was observed. To minimize the risk of confusing the action potentials generated by different cells, we considered recordings where only one spontaneously active neuron was present and where the signal-to-noise ratio was >5. Last, we limited our attention to cells generating primarily negative spikes followed by a slower positive potential, the most frequently encountered spike shape in extracellular recordings. Spike duration was defined as the interval between the negative and positive peaks because this method proved useful to distinguish principal cells from interneurons in the neocortex (Barthó et al. 2004) and hippocampus (Csicsvari et al. 1999) where, in simultaneous extra- and intracellular recordings, a close correspondence was found between this parameter and the duration of intracellularly recorded action potentials.

Figure 3 shows examples of spikes recorded at seven different locations around a representative cell with a slow (Fig. 3A) and a fast time base (Fig. 3B). Throughout the distance test, the spike amplitude remained well above noise and firing rates remained stable. The left inset of Fig. 3B plots spike duration (left axis) and amplitude (right axis) as a function of distance from the original recording site. In this cell, spike durations changed by as much as 45% from the first (black line) to the last recording site (red line). Overall, depending on the electrode position relative to recorded cells, spike durations varied by as much as 45.5 ± 4.5% (range 17–66%) of the longest measured value (1.5 ± 0.67 ms). Thus these observations imply that depending on the position of the recording electrode, one can expect maximal within-cell variations of spike durations of about 0.70 ms.

Next, we compared within-cell variations in spike durations arising from electrode position to the variability seen between different neurons. With respect to firing rates, we compared the subset of neurons that were physiologically identified as projection cells to the group of unidentified neurons that presumably included a higher proportion of local-circuit cells. However, measuring spike durations in extracellular recordings is not trivial because spike shapes vary. Although most cells generated primarily negative-going action potentials (72.2%, as in Fig. 3), some generated primarily positive-going spikes (18.1%), and others positive-negative ones (9.7%). Importantly, the particular spike shape was not a specific property of each neuron because it

**Fig. 3.** Spike duration varies depending on the microelectrode position with respect to the recorded cell. **A:** examples of action potentials recorded at various positions (numbers) around the same cell. Initial recording is marked 0 µm. Electrode was withdrawn gradually ≤110 µm from the original site. It should be noted that, even though the electrode was moved 110 µm, the total travel distance between the electrode tip and recorded cell was probably lower because the movement of the electrode likely caused the tissue to move with it. **B:** graph plotting spike duration (left axis, filled circles) and amplitude (right axis, empty squares) as a function of electrode distance from original site (x-axis). Changes are illustrated with an expanded superimposition of the spikes recorded at these various positions. Values marked by the red and black circles in the graph on the left, respectively, correspond to the spikes drawn with red and thick black traces. **Bottom right:** scheme showing hypothesized trajectory of the electrode in relation to the recorded cell. Because spikes recorded toward the end of the trajectory (red circle) were briefer and exhibited a more obvious break between the initial segment (IS) and somatodendritic (SD) components than those recorded at the original site (black circle), we presume that the thinner spikes were recorded in proximity to the initial axonal segment, whereas the longest one was recorded in the dendrites. That initial segment spikes are generally of lower amplitude than somatodendritic spikes was established in experiments where simultaneous extra- and intracellular recordings of action potentials were performed (Terzuolo and Araki 1961).
could change from one type to another when the electrode was moved around the recorded cell (Fig. 4A).

This variability in spike shapes led us to measure spike durations in two ways. In the first approach, spike durations were measured as in the distance test (interval between the negative and positive peaks for neurons generating primarily negative action potentials, Fig. 4B1; interval between positive and negative peaks for cells generating primarily positive spikes, Fig. 4B2). In the second approach, spike duration was defined as the interval between the initial change from baseline to return to baseline for both spike shapes, D and E: frequency distribution of spike durations as estimated with the first (D) and second (E) method for measuring spike duration. Different symbols are used depending on the identity of the cells: red circles for physiologically identified projection cells, black circles for unidentified cells, empty circles for cells presumed to be interneurons because of their high spontaneous firing rates (>7 Hz). Abbreviation: X, average spike duration.

Figure 4, D and E compares the distribution of spike durations as measured with the two methods in the entire sample of BL neurons (Fig. 4, D1 and E1), in the subset of identified projection cells (Fig. 4, D2 and E2), and in the unidentified neurons (Fig. 4, D3 and E3). The two methods yielded different ranges of values with a correlation coefficient of $r = 0.65$. However, in both cases the distributions ranged widely, with considerable overlap between projection cells and unidentified neurons. The difference in average spike durations (listed in the top right corner of each graph) between projection cells and unidentified neurons did not reach significance with either method ($t$-test, $P > 0.05$) and qualitatively identical results were obtained with different filtering settings (1 Hz to 3 kHz vs. 100 Hz to 10 kHz). Importantly, no correlation was observed between spike duration and firing rate with either method (Fig. 4F). However, most of the cells with extremely high firing rates did have spike durations at the lower end of the distributions (empty circles in Fig. 4F).

To test whether taking into account the entire spike shape rather than a particular attribute (such as duration) would better differentiate between cell types, we performed principal-component analysis (PCA) on our sample. Previously, PCA was used to search for better classification criteria than the immediately observable data points, solving the problem of reducing...
In some structures, extracellular data can be used to distinguish different cell types

In a number of cortical areas and subcortical structures, it was reported that different cell types have easily distinguishable firing properties. In the thalamus, for instance, cortically projecting and reticular neurons can be distinguished by their bursting behavior. Thalamocortical cells generate bursts with interspike intervals of progressively increasing durations, whereas reticular thalamic neurons produce bursts where successive interspike intervals initially show a progressive decrease and then a gradual increase in duration (Domich et al. 1986). Unfortunately, this criterion cannot be used in the amygdala because bursting projection cells account for a minority of projection neurons (Paré and Gaudreau 1996) and local-circuit cells with an indistinguishable burst-firing pattern exist in the BL nucleus (Rainnie et al. 1993).

Other classification schemes have been based on the differential responsiveness of neurons to particular drugs. In the substantia nigra, for instance, physiologically identified nigrostriatal neurons were reported to be inhibited by D2 receptor agonists, whereas other types of nigral neurons were unresponsive (Hainsworth et al. 1991). This differential responsiveness coincided with distinct electrophysiological signatures such as long-duration action potentials (>2.5 ms) and low firing rates in nigrostriatal cells compared with spikes of short-duration and elevated basal discharge rates in presumed interneurons or thalamically projecting cells (Deniau et al. 1978; Grace and Bunney 1983; Guyenet and Aghajanian 1978; Hainsworth et al. 1991; Paladini and Tepper 1999; Tepper et al. 1987). However, to the best of our knowledge, there is no well-established pattern of pharmacological responsiveness that could be used to distinguish BL projection cells and interneurons. Besides, routinely conducting such tests would be impractical.

In the hippocampus, where the position of recorded neurons with respect to the pyramidal layer is already a strong indication of their identity, it was reported that a combination of extracellular properties including spike duration, firing rate, and pattern could be used to reliably distinguish pyramidal cells from interneurons (e.g., see Csicsvari et al. 1999; Fox and Ranck 1975). However, this task is more difficult in the amygdala because interneurons and projection cells are intermingled and their dendrites are randomly oriented.

A possible solution to this problem was proposed by Buzsáki and colleagues (Barthó et al. 2004). By performing high-density neuronal recordings with silicon probes in layer 5 of the somatosensory cortex, this group examined the functional interactions between simultaneously recorded neurons and identified a subset of cells whose activity was negatively correlated to that of simultaneously recorded neurons. Such cells were regarded as putative GABAergic interneurons, whereas cells with positively correlated activity were classified as pyramidal neurons. The spike shapes of these presumed interneurons and projection cells were then used as templates to classify all recorded cells. Spike duration (defined as the interval between the negative and positive peaks, as in the present study) measured from unfiltered data (1 Hz to 5 kHz) was found to be the most reliable criterion to distinguish the two cell types.
Previous attempts to distinguish BL projection cells versus interneurons with extracellular data

Although many investigators have conducted extracellular recordings in the BL nucleus, to the best of our knowledge, only two groups have proposed criteria to distinguish projection cells from interneurons (Paré and Gaudreau 1996; Rosenkranz and Grace 1999, 2001). Both classifications were based on firing rate. In the classification of Rosenkranz and Grace (1999, 2001), neurons with firing rates < 0.5 Hz were classified as projection cells, whereas neurons with higher discharge rates where classified as interneurons. This bipartite classification of firing rate was reported to coincide with a bimodal distribution of spike durations (Rosenkranz and Grace 1999, 2001) where projection cells generated spikes of longer duration than interneurons. In contrast, Paré and Gaudreau (1996) proposed a cutoff firing rate of 10 Hz but did not examine the relation between firing rate and spike durations.

Applied to the present sample of BL neurons, the criteria proposed by Rosenkranz and Grace would have led to the erroneous classification of many projection cells as interneurons. Indeed, although the present study and a previous one in unanesthetized animals (Paré and Gaudreau 1996) reported that many projection cells fire at extremely low rates, more than half of them had firing rates > 0.5 Hz, with some firing at rates as high as 6.8 Hz. Also, spike durations were not distributed bimodally in our sample and several physiologically identified projection cells had relatively short spike durations. Consistent with this, the sample of BL cells described by Rosenkranz and Grace (1999) contained a high proportion of presumed interneurons, roughly threefold higher than that expected on the basis of counts of GABA immunoreactive neurons in the rat BL nucleus (Mascagni and McDonald 2003).

However, methodological differences might account for some of the discrepancies between these studies. First, Rosenkranz and Grace (1999, 2001) used a different anesthetic (chloral hydrate) that might have reduced the excitability of projection cells. Second, our study was conducted in cats, whereas Rosenkranz and Grace (1999, 2001) used rats. Given that the rat BL nucleus was reported to contain a higher proportion of principal cells with rapid spike frequency adaptation (Sah et al. 2003), this fact might also explain the higher proportion of principal cells with low firing rates in the studies reported by Rosenkranz and Grace (1999, 2001). In contrast, there is no evidence in the literature to explain the extremely high proportion of fast-spiking cells they observed. Although the percentage of GABAergic interneurons in the cat amygdala is unknown, observations in primates (McDonald and Augustine 1993) suggest it increases with phylogeny, as was observed in the thalamus (Arcelli et al. 1997; Winer and Larue 1996) and cortex (Fairen et al. 1984; Jones 1993; Rakic 1975).

Misclassification using firing rates and spike durations

In the present study, comparing the distributions of firing rates in physiologically identified versus unidentified BL neurons revealed only one nonoverlapping region: neurons with firing rates > 7 Hz (seen only in the unidentified neurons). It is therefore probable that cells with such high firing rates are interneurons. This contention is supported by the fact that most of these cells had spike durations at the low end of the distributions. Similarly, there was much overlap between the distribution of spike durations seen in physiologically identified projection cells and unidentified neurons. As mentioned earlier, neurons with high firing rates did tend to generate spikes of short duration, but so did many projection cells. Although this seems to contradict earlier intracellular and patch-clamp studies (see references in INTRODUCTION), we believe the discrepancy reflects the fact that high-impedance extracellular electrodes can be located at any point along the soma, dendrites, or axons of recorded cells, whereas intracellular and patch-clamp measurements are typically obtained from the soma. Consistent with this, we observed that spike shapes and durations were not a fixed property of each cell but that they varied depending on the electrode position with respect to the recorded cell.

Thus our results suggest that the only conclusive way to identify projection cells remains antidromic activation from BL projection sites. By reason of the fact that projection cells account for most BL neurons, a majority of unidentified neurons, especially those with low firing rates, are likely projection cells. In contrast, instances of misclassifications will likely be higher for interneurons. Using a cutoff of 7 Hz on our data implies that only about 5% of recorded cells were interneurons. This figure is roughly half the incidence expected on the basis of previous immunohistochemical studies, even ignoring the fact that extracellular recordings are biased toward cells with high firing rates. These considerations lead us to conclude that with a cutoff firing rate of 7 Hz, the rate of false positive identification of fast-spiking neurons is probably low, but that the rate of false negatives is probably very high, around 50%. This high rate of false negatives might be explained by the fact that there are multiple physiological subtypes of parvalbumin-positive interneurons in the BL nucleus. In support of this contention, Woodruff and Sah (2005) reported that > 20% of parvalbumin-positive BL interneurons have a regular firing pattern that is indistinguishable from that of projection cells.

**FIG. 6.** Estimated probability that a BL cell is a projection cell or interneuron depending on its firing rate. Exponential curves were fitted to the frequency distribution of firing rates among projection cells and divided by the number of recorded cells firing at each frequency. Thus the black line represents the probability that a cell at a particular firing rate is a projection cell. Dashed line is the inverse of the solid line and thus an estimate that cells with a particular firing rate are interneurons.
except for the short duration of the action potentials they generate.

In an attempt to describe the probability that a cell with a particular firing rate is a projection cell or an interneuron, we fitted exponential curves to the distribution of firing rates for both identified projection cells and the whole neuronal population in our data. Figure 6 (continuous line) shows the ratio of known projection cells to the entire population at all encountered frequencies. The one’s complement of this probability curve yields an estimate (dashed line) of the likelihood that neurons with particular firing rates are interneurons. On this basis, we propose that the optimal approach to obtain pure samples of projection cells and interneurons is to use two firing cutoffs: ≤1 Hz for projection cells and ≥7–8 Hz for fast-spiking interneurons. Indeed, the probability functions of Fig. 6 suggest that, at a cutoff frequency of 7.4 Hz, the probability of incorrectly identifying an interneuron as a projection cell is <5%. The incidence of misclassifications should be very low with this approach, especially if used in combination with two spike duration cutoffs (≥0.7 ms for projection cells and ≤0.5 ms for fast-spiking interneurons). The drawback of this approach is that it forces investigators to ignore many of the recorded cells. On the other hand, one can be confident about the identity of recorded neurons. A challenge for future studies will be to test the validity of this approach by directly identifying the morphology of presumed interneurons using juxtacellular labeling (Pinault 1996) of BLA neurons with neurobiotin.

ACKNOWLEDGMENTS

We thank J. Tepper and members of the Pare lab for comments on an earlier version of this manuscript.

GRANTS

This work was supported by National Science Foundation Grant 0208712 and by National Institute of Mental Health (NIMH) Grants R01MH-073610-01 to D. Pare and NIMH National Research Service Award Grant 1 F31 MH-076415-01 to E. Likhtik. We thank J. Tepper and members of the Pare lab for comments on an earlier version of this manuscript.

REFERENCES


Lang EJ and Paré D. Synthetic and synapticly activated intrinsic condu-


Lemon R. Methods for Neuronal Recordings in Conscious Animals. Chich-


Paladini CA and Tepper JM. GABA(A) and GABA(B) antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. *Synapse* 32: 165–176, 1999.


