THE AMYGDALOSTRIATAL PROJECTION
An Analysis of Synaptic Inputs to GABAergic Interneuron Subtypes

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INTRODUCTION

The striatum receives massive inputs from the cortex, thalamus and midbrain, and is also afferented by serotonergic and noradrenergic cells groups, and the globus pallidus (Heimer et al. 1995; Parent, 1990, for reviews). Recent studies have revealed a dense amygdalostriatal projection which is centered upon the nucleus accumbens, but which topographically distributes over a large striatal area excluding the sensorimotor sector (Wright and Groenewegen, 1995; Johnson et al., 1994a,b; McDonald et al, 1991; Kita and Kitai, 1990; Russchen and Price, 1984; Groenewegen et al., 1982,1980; Kelley et al., 1982; DeFrance et al., 1980; Krettek and Price,1978; Dafny et al. 1975; de Olmos, 1972). In rodents, the amygdalostriatal projection arises mainly from the basolateral nuclear complex. Other amygdala nuclei contributing to the projection include the accessory basal and cortical nuclei, the amygdalohippocampal transitional area, and the nucleus of the lateral olfactory tract. These inputs are excitatory and use glutamate as a neurotransmitter (Callaway et al., 1991; Robinson and Beart, 1988; Christie et al., 1987; Fuller et al., 1987). As is true for cortical projections to the dorsal and ventral striatum (Sesack and Pickel, 1992; Totterdell and Smith, 1989; Dubé et al, 1988; Frotscher et al., '81; Somogyi et al., '81; Kemp and Powell, '71), amygdala projections terminate mainly on dendritic spines of medium spiny neurons (Johnson et al., 1994a,b; Kita and Kitai, 1990).

The vast majority of striatal neurons are medium spiny projection cells. The interneurons may play an important role in modulation of striatal output and in regulation of the activation state of the striatum (Smith and Bolam, 1990 for review). Interneurons in the
striatum include cholinergic, somatostatinergic, and GABAergic cell types. The aspiny GABAergic interneurons (Cowan et al., 1990; Kita and Kitai, 1988; Bolam et al. 1983, 1985) are composed of at least two subclasses based on differential expression of calcium binding proteins (Bennett and Bolam, 1994, 1993; Kawaguchi, 1993; Kubota et al., 1993; Cowan et al., 1990; Kita et al., 1990). One subtype expresses calretinin (CR), the other expresses parvalbumin (PV). Somatostatinergic neurons may represent a third subclass of GABAergic interneuron (Kubota et al., 1993). The paucity of information on amygdala inputs to striatal interneurons, and recent demonstration of the potential importance of GABAergic interneurons as a putative substrate for feed-forward inhibition (Bennett and Bolam, 1994, Jaeger et al., 1994; Lapper et al, 1992; Pennartz and Kitai, 1991; Kita et al, 1990), led us to further investigate amygdaloid inputs to GABAergic interneurons of the ventral striatum. In order to investigate possible differences in afferent inputs to GABAergic interneuron subtypes, we examine the amygdalostriatal projection at the electron microscopic level using double-immunohistochemistry for revelation of anterograde tracer and CR or PV (Llewellyn-Smith '93; Smith and Bolam '92).

METHODS

20 adult female (240-270 g) Sprague Dawley rats were anaesthetized with a combination of ketamine hydrochloride (10mg/kg, i.p.) and xylazine (5-10mg/kg, i.p.). Unilateral stereotactic injections of 5% biotinylated dextranamine (BDA, Molecular Probes) in 0.9% saline were made in the basolateral complex of the amygdala. Iontophoretic injections were made by applying 7-10 μA pulses (7s on, 7 s off) of direct current over 20-30 minutes through a silver wire immersed in a glass micropipette (internal tip diameter 20-50 μm) containing BDA. Pressure injections were made by injection of 0.5 μl of BDA over 15 minutes using a 2 μl Hamilton syringe.

After a 6-14 day survival period, rats were given a lethal dose of ketamine/xylazine and perfused transcardially. The initial rinse was either with 100ml of cold heparinized saline or with cold oxygenated Ringer’s solution. The fixative solution was perfused over a period of 20 minutes and consisted of 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate buffer (0.1M, pH 7.4). Brains were removed and postfixed in the fixative solution overnight. 50-60 μm thick sections were cut on a vibrating microtome in the coronal plane and collected in PBS (0.1M, pH 7.4).

BDA was localized by incubating sections overnight at room temperature in avidin-biotin-peroxidase complex (ABC, Vector Labs; 1:100) containing 0.1% Triton-X-100 and 1% bovine serum albumin (BSA, Sigma) dissolved in PBS. The labeling was revealed by exposure for 10-20 minutes to a solution containing diaminobenzidine (DAB, 25 mg/100ml (Sigma)), imidazole (1 ml/100 ml), and 0.006% hydrogen peroxide dissolved in Tris buffer (pH 7.6). After rinsing extensively in PBS, the sections were mounted out of distilled water, air-dried, dehydrated in a graded series of alcohols, cleared in xylene and coverslipped with Permount (Fisher). At the level of the injection site, sections adjacent to BDA sections, were processed for acetylcholinesterase histochemistry (Geneser-Jensen and Blackstad, '71). In 4 animals, the injection site corresponded to the acetylcholinesterase-rich basolateral complex of the amygdala. Other sections from these animals were processed for either light or electron microscopic double-labeling immunohistochemistry (Smith and Bolam, '92).

Sections for light microscopy were treated for 20 minutes in sodium borohydride (1% in PBS), preincubated for one hour at room temperature in a PBS solution containing 2% BSA and 0.1% Triton-X-100, washed repeatedly in PBS, incubated overnight in ABC, and then processed using DAB as a chromogen as described above. Sections were then incubated overnight at room temperature in primary antibody dissolved in PBS containing.