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Research Report

Identification and distribution of projections from monoaminergic and cholinergic nuclei to functionally differentiated subregions of prefrontal cortex

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ABSTRACT

The prefrontal cortex (PFC) is implicated in a variety of cognitive and executive functions and is composed of several distinct networks, including anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), and orbitofrontal cortex (OFC). These regions serve dissociable cognitive functions, and are heavily innervated by acetylcholine, dopamine, serotonin and norepinephrine systems. In this study, fluorescently labeled retrograde tracers were injected into the ACC, mPFC, and OFC, and labeled cells were identified in the nucleus basalis (NB), ventral tegmental area (VTA), dorsal raphe nucleus (DRN) and locus coeruleus (LC). DRN and LC showed similar distributions of retrogradely labeled neurons such that most were single labeled and the largest population projected to mPFC. VTA showed a slightly greater proportion of double and triple labeled neurons, with the largest population projecting to OFC. NB, on the other hand, showed mostly double and triple labeled neurons projecting to multiple subregions. Therefore, subsets of VTA, DRN and LC neurons may be capable of modulating individual prefrontal subregions independently, whereas NB cells may exert a more unified influence on the three areas simultaneously. These findings emphasize the unique aspects of the cholinergic and monoaminergic projections to functionally and anatomically distinct subregions of PFC.

1. Introduction

The PFC is associated with several higher order cognitive functions such as rule-based and goal-directed behaviors, working memory, decision-making and reward seeking (Brown and Bowman, 2002; Dalley et al., 2004; Furuyashiki and Gallagher, 2007; Fuster, 2000; Ongur and Price, 2000; Passetti et al., 2002; Robbins, 2000). The connectivity and intrinsic organization of this region of the brain is optimal for its role in abstract behavioral and executive processes (Dalley et al., 2004; Fuster, 2000; Hoover and Vertes, 2007; Passetti et al., 2002). The PFC is composed of several anatomically and functionally distinct subregions, including OFC, mPFC, and ACC. In the rodent, OFC is implicated in reversal learning and

Abbreviations: OFC, Orbitofrontal cortex; mPFC, medial prefrontal cortex; ACC, anterior cingulate cortex; NB, nucleus basalis; VTA, ventral tegmental area; DRN, dorsal raphe nucleus; LC, locus coeruleus; VAChT, vesicular acetylcholine transporter; TH, tyrosine hydroxylase; 5HT, serotonin

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lower order sensory discriminations (Dalley et al., 2004; Furuyashiki and Gallagher, 2007; Kolb et al., 2004; Murray et al., 2007; Rushworth et al., 2009; Schoenbaum et al., 2007; Sul et al., 2010), while mPFC is involved in higher order sensory-based discriminations, behavioral flexibility and sustained attention (Dalley et al., 2004; Floresco et al., 2008; McGaughy et al., 2008; Newman et al., 2008), and ACC is implicated in behavioral impulse control and regulation (Bussey et al., 1997). Importantly, functional, and to a lesser degree, anatomical, homology exists between rodent and human PFC (Dalley et al., 2004). These regions are each unique in their afferent and efferent connections (Dalley et al., 2004; Hoover and Vertes, 2007); however, several ascending neuromodulatory pathways all converge in these regions to regulate network activity. The purpose of this study was to identify the organization and distribution of cells in NB, VTA, DRN and LC that project to functionally and anatomically distinct subregions of PFC.

The NB is the primary source of cholinergic input to the cerebral cortex (Sarter and Bruno, 2000; Wenk, 1997) and has been implicated in arousal, learning, attention and memory (McGaughy et al., 2000; McGaughy and Sarter, 1998, 1999; Nieto-Escamez et al., 2002; Sarter and Bruno, 2000; Wenk, 1997). A rough topography has been identified in the primate analog of NB, nucleus basalis of Meynert, such that anteromedial portions of the nucleus project to the medial surface of the cortex, anterolateral regions project to frontal and parietal cortices and amygdala, intermediate regions project to prefrontal, insular and posterior parietal cortices, and caudal portions project to the superior and temporal cortex (Pang et al., 1993). This nucleus is less well defined in rodent and its cholinergic projection neurons are more scattered (Sarter and Bruno, 2000; Wenk, 1997); however, it similarly stains intensely for cholinergic markers, is situated in roughly the same region of the brain, and has also been implicated in the modulation of higher order cognitive processes (Lehmann et al., 1980; McGaughy and Sarter, 1998; 1999; Nieto-Escamez et al., 2002; Sarter and Bruno, 2000; Wenk, 1997). This group of cells has also been given the designation Ch4 by Mesulam and colleagues (Mesulam et al., 1983).

The VTA is similarly involved in several higher order cognitive processes such as reward seeking and working memory (Carr et al., 1999; Chambers et al., 2010; Greene, 2006; Grimm et al., 2004; Li et al., 2009; Pang et al., 1993; Schultz, 1998; Vucetic et al., 2010; Wang et al., 2010). Previous accounts of the VTA projection system indicate that its efferents do not collateralize extensively (Loughlin and Fallon, 1984; Sobel and Corbett, 1984). Furthermore, these cells have been shown to be topographically ordered with respect to their projection targets (Beckstead et al., 1979; Carter and Fibiger, 1977; Fallon et al., 1978; Fallon and Loughlin, 1982; Fallon and Moore, 1978a, 1978b; Loughlin and Fallon, 1984; Sobel and Corbett, 1984) such that medial cell groups innervate more medial and rostral structures, while laterally positioned cells innervate more lateral and caudal structures (Loughlin and Fallon, 1984).

The DRN is one of several midbrain serotonergic nuclei, and the primary source of serotonin to the forebrain. It is involved in the regulation of mood, sleep and waking cycles (Mamounas and Molliver, 1988; Moore and Halaris, 1975; Moore et al., 1978; O'Hearn and Molliver, 1984). The DRN displays a rough topographical organization (Abrams et al., 2004; Vertes, 1991) such that more rostral structures are innervated by more rostral portions of DRN whereas caudal structures receive input from more caudal clusters of cells (Abrams et al., 2004). Cortical structures receive input primarily from cells located along the midline and dorsal to the medial longitudinal fasciculus, whereas subcortical structures receive projections from cells located in the lateral wings (Kirifides et al., 2001; O'Hearn and Molliver, 1984; Van Bockstaele et al., 1993; Villar et al., 1988; Waterhouse et al., 1986, 1993). Furthermore, it has been shown that DRN cells collateralize more extensively to forebrain structures than do those projecting from VTA (Sobel and Corbett, 1984; Van Bockstaele et al., 1993) and that axons emanating from individual DRN neurons tend to send collaterals to multiple functionally related targets simultaneously (Abrams et al., 2004; Simpson et al., 1997; Van Bockstaele et al., 1993). The projections from DRN to various subregions of PFC have not been characterized (Tables 1–4).

The LC is the only source of norepinephrine-containing fibers to the PFC (Berridge and Waterhouse, 2003; Sara, 2009) and, in addition, exerts a widespread influence on neuronal circuitries involved in sensory processing, motor behavior, arousal and cognitive processes (Berridge and Waterhouse, 2003; Cain et al., 2011; Devilbiss et al., 2006; Devilbiss and Waterhouse, 2000, 2004; Hurley et al., 2004; McGaughy et al., 2008; McGaughy and Sarter, 1998; Moxon et al., 2007; Newman et al., 2008; Sara, 2009). Previous reports that describe LC anatomy suggest that this nucleus is highly divergent with only minimal efferent topographic organization (Fallon and Loughlin, 1982; Loughlin et al., 1982; Waterhouse et al., 1983, 1993), although some LC cells send axon collaterals to multiple target structures along the same sensory pathway (Simpson et al., 1997, 1999, 2006). However, the nature of

Table 1 – Raw counts of retrogradely labeled cells in NB projecting to all possible combinations of terminal fields.

Case	OFC	mPFC	ACC	OFC+mPFC	OFC+ACC	mPFC+ACC	All	Total
2	18	14	10	22	13	16	37	130
4	8	14	6	9	9	16	19	81
5	12	16	9	9	9	6	18	79
6	7	9	5	12	10	19	20	82
8	8	12	10	15	12	13	16	86
9	18	21	12	9	7	16	19	102
10	21	26	15	18	8	14	20	122
Cumulative (%)	92 (13.5%)	112 (16.4%)	67 (9.8%)	94 (13.8%)	68 (10.0%)	100 (14.7%)	149 (21.8%)	682 (100%)