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THE PARABRACHIAL NUCLEUS IS A CRITICAL LINK IN THE TRANSMISSION OF SHORT LATENCY NOCICEPTIVE INFORMATION TO MIDBRAIN DOPAMINERGIC NEURONS

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Abstract—Many dopaminergic neurons exhibit a short-latency response to noxious stimuli, the source of which is unknown. Here we report that the nociceptive-recipient parabrachial nucleus appears to be a critical link in the transmission of pain related information to dopaminergic neurons. Injections of retrograde tracer into the substantia nigra pars compacta of the rat labelled neurons in both the lateral and medial parts of the parabrachial nucleus, and intra-parabrachial injections of anterograde tracers revealed robust projections to the pars compacta and ventral tegmental area. Axonal boutons were seen in close association with tyrosine hydroxylase-positive (presumed dopaminergic) and negative elements in these regions. Simultaneous extracellular recordings were made from parabrachial and dopaminergic neurons in the anaesthetized rat, during the application of noxious footshock. Parabrachial neurons exhibited a short-latency, short duration excitation to footshock while dopaminergic neurons exhibited a short-latency inhibition. Response latencies of dopaminergic neurons were reliably longer than those of parabrachial neurons. Intra-parabrachial injections of the local anasethetic lidocaine or the $GABA_A$ receptor antagonist muscimol reduced tonic parabrachial activity and the amplitude (and in the case of lidocaine, duration) of the phasic response to footshock. Suppression of parabrachial activity with lidocaine reduced the baseline firing rate of dopaminergic neurons, while both lidocaine and muscimol reduced the amplitude of the phasic inhibitory response to footshock, in the case of lidocaine sometimes abolishing it altogether. Considered together, these results suggest that the parabrachial nucleus is an important source of short-latency nociceptive input to the dopaminergic neurons. © 2010 IBRO. Published by Elsevier Ltd.

Key words: nociception, extracellular recording, tract tracing, rat.

Dopamine-mediated transmission has been implicated in a number of human clinical disorders as well as in a wide range of normal brain functions. Typically, dopaminergic (DA) neurons exhibit a highly stereotyped, short latency (<100 ms), short duration (~100 ms) population response to unpredicted stimuli in a variety of modalities that are salient by virtue of their novelty, intensity or reward value (Freeman and Bunney, 1987; Horvitz et al., 1997; Schultz, 1998). Dopaminergic neurons also respond to noxious stimuli in a wide range of species, including the rat (e.g. Coizet et al., 2006), rabbit (Guarraci and Kapp, 1999) and monkey (e.g. Schultz and Romo, 1987). In the rat, noxious stimuli produce a short-latency increase, or more commonly decrease, in discharge frequency (Tsai et al., 1980; Maeda and Mogenson, 1982; Mantz et al., 1989; Gao et al., 1990; Ungless et al., 2004; Coizet et al., 2006).

While much is known about many aspects of the ascending dopamine systems, surprisingly little is known about the sensory inputs that phasically modulate their activity. We have recently shown that a subcortical visual structure, the midbrain superior colliculus (SC), is the primary, if not the exclusive, source of short-latency visual input to midbrain DA neurons (Comoli et al., 2003; Dommett et al., 2005), possibly mediated in part by the tectonigral projection—a direct projection from the SC to the substantia nigra pars compacta (SNPc) and ventral tegmental area (VTA), which innervates DA neurons in these regions (Comoli et al., 2003; McHaffie et al., 2006; May et al., 2009). However, although the SC contains neurons which respond to noxious stimuli (e.g. Stein and Dixon, 1979), in contrast to vision, it does not appear to transmit pain-related information to DA neurons (Coizet et al., 2006).

Although the source of the afferent inputs which relay pain-related information to DA neurons is still uncertain, during our retrograde anatomical work on the tectonigral projection, which involved the placement of tracer injections in the SNPc, we noticed numerous retrogradely labelled cells in the mesopontine parabrachial nucleus (PBN). The PBN is a major central target for ascending nociceptive information from the spinal cord (Hylden et al.,

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Abbreviations: ANOVA, analysis of variance; BDA, biotinylated dextran amine; BSA, bovine serum albumin; DA, dopaminergic; DAB, diaminobenzidine; FLI, fos-like immunoreactivity; NHS, normal horse serum; PB, phosphate buffer; PBN, parabrachial nucleus; PBS, phosphate buffered saline; PHA-L, *Phaseolus vulgaris* leukoagglutinin; PPTg, pedunculoponting tegmental nucleus; PSTH, peri-stimulus time interval histogram; RMTg, rostromedial tegmental nucleus; SD, standard deviation; SNPc, substantia nigra pars compacta; SNPr, substantia nigra pars reticulata; TH, tyrosine hydroxylase; TX, Triton X 100; VTA, ventral tegmental area.

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1989; Craig, 1995; Klop et al., 2005), which raises the possibility that the PBN may provide nociceptive signals to DA neurons. This was investigated initially by using tract tracing experiments to confirm the existence of a direct parabrachio–nigral projection and explore its properties. Following these, we used electrophysiological procedures to examine the effects of chemical inactivation of the PBN (using the local anaesthetic lidocaine or the $GABA_A$ receptor antagonist muscimol) on the phasic responses of DA neurons to noxious stimuli.

EXPERIMENTAL PROCEDURES

All aspects of these studies were performed with Home Office approval under section 5(4) of the Animals (Scientific Procedures) Act 1986, and experimental protocols received prior approval from the Institutional Ethics Committees.

Anatomical experiments

Surgical preparation. For the retrograde and anterograde tract tracing experiments, 12 male Hooded Lister rats (398–672 g) were anaesthetized with an i.p. injection of a mixture of ketamine (Ketaset, 0.765 ml/kg) and xylazine (Rompun, 1.1 ml/kg) and mounted in a stereotaxic frame (David Kopf Instruments, Tuajanga, CA, USA) with the skull level. Body temperature was maintained at 37 °C with a thermostatically controlled heating blanket.

Retrograde tracer injections. In the first group of rats ($n=4$), the retrograde tracer fluorogold (Fluorochrome LLC, Denver, CO, USA) was injected into the SNPc (5.2–6.04 caudal to bregma, 1.4–2.6 mm lateral to midline, 7.3–8.2 mm below the brain surface) as a 4% solution in distilled water (45–100 nl) via a glass micropipette using a compressed air injection system. As described in a previous paper (Coizet et al., 2007), these injections were made under electrophysiological guidance to improve the successful placement of the tracer. Briefly, the glass pipette was joined to a Parylene-C coated tungsten electrode (2 MΩ; A-M Systems Inc., Carlsborg, WA, USA) and the assembly lowered into the ventral midbrain until the electrophysiological record showed an absence of activity (usually at a depth of around 8.0 mm), corresponding to the medial lemniscus. Shortly after, the record typically revealed the presence of fast firing activity characteristic of neurons in the substantia nigra pars reticulata. Tracer injections were made as soon as this fast activity was encountered.

Retrograde tracer histology and analysis. After allowing 7 days for the transport of tracers, animals were re-anaesthetized with pentobarbitone and perfused transcardially with warm saline (40 °C) followed by 4% paraformaldehyde in phosphate buffer (PB) (pH 7.4). The brains were placed in 10% formalin for 4 h before being cryoprotected by immersion in sucrose solution (20% in 0.1 M PB) overnight. The next day, coronal sections (30 μm) were cut on a freezing microtome and collected directly onto slides, allowed to dry in a light protected box and coverslipped in DPX.

The injection sites and retrogradely labelled cells in the PBN were examined with a fluorescent microscope equipped with episcopic illumination (Nikon Eclipse E800M, Kingston-upon-Thames, UK) and UV excitation filter (330–380 nm). The location of retrogradely-labelled neurons was plotted on three coronal sections through the PBN separated by ~0.5 mm (equivalent to 8.8, 9.3 and 9.8 mm caudal to bregma, corresponding to anterior, central and posterior regions of the PBN respectively). A series of digital images (magnification×100) were taken using an RT Colour Spot camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) and imported into a graphics program (Macromedia Freehand, Adobe, San Jose, CA, USA) where they were montaged. Quantitative differences in cell counts within the PBN were assessed by repeated measures ANOVA (factors Laterality [levels: Lateral, Medial] and anterior–posterior position [levels: Anterior, Central, Posterior; accepted significance level $P<0.05$, 2 tailed).

Anterograde tracer injections. In a second group of rats, single injections of the anterograde tracers biotinylated dextran amine (BDA, Sigma-Aldrich; $n=5$) or *Phaseolus vulgaris* leucoagglutinin (PHA-L, Vector Laboratories, Peterborough, UK; $n=3$) were made into the PBN. An angled approach was used, with the injector tilted caudally by 35°, entering the brain at 11.2 mm caudal to bregma and 2.0 mm lateral to midline, after which it was inserted 6.0 mm below the brain surface. BDA (10% in phosphate buffer; PB) was pressure ejected in volumes of 30–90 nl via a glass micropipette (20 μm diameter tip) using a compressed air injection system, while PHA-L was ejected iontophoretically (5 μA anodal current applied to a 2.5% solution in PB, 7 s on/off for 15–20 min). After allowing 7 days for the transport of tracers, animals were re-anaesthetized with pentobarbitone and perfused transcardially with saline followed by 4% paraformaldehyde in PB (pH 7.4). The brains were placed immediately in 10% formalin for 4 h before being cryoprotected by immersion in sucrose solution (20% in 0.1 M PB) overnight. The next day, two series of coronal or sagittal sections (30 μm) were cut on a freezing microtome and collected in 0.1 M PB. Both series were processed to reveal the anterograde tracers, however the second series was subjected to an additional step, in which they were processed for tyrosine hydroxylase (TH).

Anterograde tracer histology and analysis. To reveal the tracers (BDA and PHA-L), free-floating sections were washed with 0.1 M PB followed by 0.1 M PB containing 0.3% Triton X-100 (PB-TX) for 30 min. For animals injected with PHA-L, the sections were incubated overnight in primary antibody solution (goat anti-PHA-L, 1:800–1,000 dilution, Vector Laboratories). The next day, sections were washed with PB-TX and incubated for 2 h in biotinylated rabbit anti-goat IgG (1:100, Vector Laboratories, in PB-TX containing 2% normal rabbit serum) for PHA-L. After 30 min of washing, all the sections from PHA-L and BDA animals were incubated in Elite Vectastain ABC reagent (Vector laboratories, 1:100 in PB-TX) for 2 h. The peroxidase associated with the tracers was revealed by reacting tissue with H_2O_2 for approximately 1 min using nickel-enhanced diaminobenzidine (DAB) as the chromogen for BDA (black reaction product), while PHA-L was revealed by incubation with VIP (Vector Laboratories) chromogen (purple reaction product). Finally, sections were washed in PB, and the first series were mounted on gelatin-coated slides, dehydrated in graded dilutions of alcohol, cleared in xylene and coverslipped using DPX.

The second series of sections were processed for TH as follows: Sections were incubated overnight with the primary mouse monoclonal antibody diluted 1:3000 (Roche Diagnostics, Lewes, UK) in 0.1 M PB-Trition-X 100 (TX) 0.3% with 1% bovine serum albumin (BSA) and 2% normal horse serum (NHS). The following day, sections were washed in 0.1 M PB and the secondary antibody, biotinylated antimouse made in horse (in a dilution of 1:1000 in 0.1 M PB-TX 0.3% with 2% NHS), was applied for 2 h. Following further washes in 0.1 M PB, the sections were exposed to the elite Vectastain ABC reagent (Vector Laboratories) diluted 1:100 in PB-TX 0.3% for 2 h. Again following washes in 0.1 M PB, immunoreactivity was revealed by exposure to VIP (Vector laboratories) for 3 min followed by several washes in 0.1 M PB to stop the reaction. Slices were then mounted onto gelled slides, dehydrated through alcohols and cleared in xylene before being coverslipped with DPX.