Functional imaging of the human dopaminergic midbrain

Düzel, E; Bunzeck, N; Guitart-Masip, M; Wittmann, B; Schott, B H; Tobler, Philippe N

Abstract: Invasive recording of dopamine neurons in the substantia nigra and ventral tegmental area (SN/VTA) of behaving animals suggests a role for these neurons in reward learning and novelty processing. In humans, functional magnetic resonance imaging (fMRI) is currently the only non-invasive event-related method to measure SN/VTA activity, but it is debated to what extent fMRI enables inference about dopaminergic responses within the SN/VTA. We consider the anatomical and functional parcellation of the primate SN/VTA and find that its homogeneity suggests little variation in the regional specificity of fMRI signals for reward-related dopaminergic responses. Hence, these responses seem to be well captured by the compound fMRI signal from the SN/VTA, which seems quantitatively related to dopamine release in positron emission tomography (PET). We outline how systematic investigation of the functional parcellation of the SN/VTA in animals, new developments in fMRI analysis and combined PET-fMRI studies can narrow the gap between fMRI and dopaminergic neurotransmission.

DOI: https://doi.org/10.1016/j.tins.2009.02.005

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-50262
Published Version

Originally published at:
DOI: https://doi.org/10.1016/j.tins.2009.02.005
Invasive recording of dopamine neurons in the substantia nigra and ventral tegmental area (SN/VTA) of behaving animals suggests a role for these neurons in reward learning and novelty processing. In humans, functional magnetic resonance imaging (fMRI) is currently the only non-invasive event-related method to measure SN/VTA activity, but it is debated to what extent fMRI enables inference about dopaminergic responses within the SN/VTA. We consider the anatomical and functional parcellation of the primate SN/VTA and find that its homogeneity suggests little variation in the regional specificity of fMRI signals for reward-related dopaminergic responses. Hence, these responses seem to be well captured by the compound fMRI signal from the SN/VTA, which seems quantitatively related to dopamine release in positron emission tomography (PET). We outline how systematic investigation of the functional parcellation of the SN/VTA in animals, new developments in fMRI analysis and combined PET–fMRI studies can narrow the gap between fMRI and dopaminergic neurotransmission.

Introduction

Dopamine modulates motivational aspects of behaviour and cognition. It profoundly regulates neural processes in reinforcement learning [1], reward seeking [2], hippocampal plasticity [3], working memory [4], decision making and economic choice [2,5], addiction [6], behavioural drive [7] and incentive motivation [8]. Dopamine is also implicated in the pathophysiology of neurological and neuropsychiatric disorders and in age-related cognitive decline [9–15]. The study of dopaminergic neurotransmission is, therefore, of key importance to a large and multidisciplinary scientific community including clinicians, cognitive neuroscientists, neuroeconomists and animal physiologists.

Our knowledge of the functional properties of dopamine neurons comes primarily from extracellular recordings of single dopamine neurons in non-human primates. Such studies suggest that ~70% of neurons respond with a phasic burst (latency and duration ~100 ms) to unpredicted liquid or food reward, to stimuli predicting such rewards and to novel and salient stimuli [1]. Neurons show substantial responses to both unpredicted liquid and food rewards but only limited responses to aversive stimuli such as air puffs and hypertonic saline. Activation by unpredicted but not by predicted rewards, together with depression by omitted but predicted rewards, suggests that phasic dopamine activity codes an error in the prediction of reward (difference between predicted and actual rewards) [1]. Reward prediction errors capture the need for learning and are important in theories of learning [16]. Thus, burst firing of dopamine neurons seems to contribute to reward processing and more specifically to reward learning, but it remains an open question whether dopamine neurons fulfil a similar role in humans.

In humans, a major challenge in studying dopamine neurotransmission and release is the experimental limitation and health risk of human single-cell recording and of molecular imaging methods such as positron emission tomography (PET). As a non-invasive, repeatable and event-related functional technique, functional magnetic resonance imaging (fMRI) has, therefore, attracted widespread attention. Indeed, it might be possible that fMRI can indirectly inform us about dopaminergic neurotransmission [17–22]. Central to this approach is that most dopaminergic projection neurons colocalize in the substantia nigra and ventral tegmental area (SN/VTA) of the midbrain and the assumption that, therefore, fMRI responses from the SN/VTA should be related to dopamine neurotransmission observed in animals and ultimately to dopamine release.

A primary source of uncertainty with fMRI of the SN/VTA is that the link between hemodynamic SN/VTA responses and dopamine firing or release is inferential and remains necessarily indirect. Our goal here is to highlight to the fMRI community which anatomical and physiological factors conceptually determine the gap between fMRI and dopaminergic neurotransmission (Box 1) and to highlight to rodent and non-human primate physiologists which anatomical and physiological answers would help to narrow that gap. First, we focus on the functional–anatomical parcellation of the SN/VTA and the species differences of this parcellation. Second, we address the
physiological mechanisms that control dopamine release from the vantage point of how these mechanisms might contribute to fMRI responses. Finally, we describe conceptual avenues for future research (we do not touch upon technical and methodological challenges of fMRI in the SN/VTA).

Anatomical location of dopamine neurons: the importance of species differences

To optimize the specificity of fMRI for dopaminergic neurons, data acquisition should ideally focus onto the part of the SN/VTA with the highest density of dopamine neurons responding to motivationally salient events such as rewards. One of the prevailing assumptions in the field is that among all dopaminergic cell groups (i.e. retrorubral field, SN and VTA; Box 2), the VTA should be specifically targeted. However, this assumption is primarily motivated by the functional–anatomical parcellation of the dopaminergic complex in rodents and does not adequately reflect anatomy and function in primates.

Inter-species comparisons show both continuity and discontinuity with respect to midbrain dopamine regions. The similarity in the distribution and cytochemistry of dopamine neurons suggests continuity between baboons and humans [23]. However, important differences exist between rodents and primates. First, positional rearrangement of SN/VTA subcompartments occurs; it is the dorsal part of the primate SN that is most representative of the rat VTA region [24] (Figure 1). Second, the ratio of dopamine to non-dopamine neurons is larger in the dopamine complex of humans than rats. In humans and non-human primates, ~75% of dopamine neurons are in the substantia nigra pars compacta (SNc), 15% in the VTA and 10% in the retrorubral field [25,26]. The corresponding amounts in the rat are ~45% for both SNc (A9 in the rat) and VTA (A10), and 10% for the retrorubral field (A8) [27]. Thus, in humans, the majority of dopamine neurons are located in the SNc, which, contrary to rodents, protrudes into the substantia nigra pars reticulata (SNr) [28] (Figure 1). Third, the human retrorubral field is more extensive than in the rat, succeeded rostrally by the human-specific midbrain reticular field (Figure 1). Together, a focus on VTA at the expense of other dopaminergic regions would neglect the majority of human dopamine neurons and miss out on human-specific fields.

Distinguishing the VTA and SNc might be particularly difficult in humans for anatomical reasons. The SNc is more continuous with the VTA in humans and primates than in rats [29]. Indeed, the borders between dopaminergic subregions are so difficult to distinguish that some anatomists subdivide dopamine neurons into dorsal and ventral tiers rather than the VTA, SNc and retrorubral nucleus [30]. However, even a simple dorso-ventral subdivision is more complete in rats than primates [31].

Likewise, a delineation of dopamine subregions based on distinct efferent projection pathways (e.g. mesolimbic, mesocortical and nigrostriatal; Box 2) seems to be difficult to achieve in humans. In non-human primates and, more notably, in humans, dopamine neurons that project to
limbic and orbitofrontal regions are not confined to the VTA but are dispersed across the SN/VTA [32–34]. These marked species differences need to be considered when interpreting human fMRI results in the light of rodent work.

### Functional topography of SN/VTA in non-human primates

Despite the absence of clear anatomical delineators in the primate mentioned earlier, functional differences might occur between dopamine cell groups. If so, the specificity of fMRI for dopaminergic responses would regionally differ within SN/VTA depending on the type of motivational event of interest (e.g. rewards, novelty and aversive events).

The literature, thus far, has not reported responses specific to VTA, SNc or retrorubral field [35–37]. This lack of reports seems to reflect a lack of robust functional specificity differences between these regions. Indeed, most studies collapse neurons from different subregions owing to their response similarities. Such response similarity suggests little scope for focussing human fMRI on subregions from a functional perspective.

However, it is possible that only the VTA contains dopamine neurons that show activations to conditioned stimuli predicting punishment [38] or to noxious stimuli [39]. The projection targets of these dopamine neurons

---

*Paul Glimcher, Takemasa Satoh, Minoru Kimura, Genela Morris, Hagai Bergman, personal communication.
might be different from dopamine neurons that activate in response to rewards. Moreover, in rats the VTA rather than the SNc might contain neurons that in choice situations code the reward value of the better of two options [40], whereas the SNc in monkeys would contain neurons that code the reward value of the chosen option [41]. Aside from these suggestions of functional dissociations, so far there is only evidence for gradual differences between dopamine cell regions. For example, responses to unpredicted reward can be stronger in the monkey VTA than the SNc and retrorubral field [42], and depressions to stimuli predicting reward omission might be stronger in the retrorubral field than in the VTA or SNc [43]. Functional gradients do not always coincide with borders of dopaminergic cell groups: in monkeys, more lateral neurons show stronger movement-related responses [44] and stronger depressions to stimuli predicting reward omission [43]. In conclusion, most functional data thus far suggest no or gradual, rather than qualitative, differences in the functions of dopaminergic SNc, VTA and retrorubral neurons in primates. However, conclusions about functional compartmentalization are sometimes difficult to reach because of sampling bias (in the rat, bias towards VTA [G. Schoenbaum, personal communication], possibly owing to a higher ratio of dopamine to non-dopamine neurons; in the monkey, bias towards SNc, possibly owing to size and accessibility). Finding functional gradients in animals could provide clues for human research and indicate a need for sophisticated fMRI techniques employing spatial priors about these gradients.

**SN/VTA activation in fMRI: input, output, inhibition?**

A key question in relating fMRI responses in the SN/VTA to dopaminergic neurotransmission is what aspect of neural responses in the SN/VTA is most likely to be represented in the fMRI signal (Figure 2). The fMRI blood oxygenation-level-dependent (BOLD) image contrast is an indirect measure of neuronal activity that is based upon changes in deoxyhemoglobin concentration in response to neuronal dynamics at the cellular and micro-circuitry level [45,46]. Co-recordings of local field potentials, multi-unit activity and fMRI signals in anesthetized [47] and awake [46] monkeys indicate that the BOLD response correlates with postsynaptic local field potentials (LFPs). To a large extent, these LFPs reflect postsynaptic membrane voltage oscillations resulting from excitatory presynaptic input and local, somatodendritic integrative processes [47]. The presynaptic inputs can originate from a different neuronal population, or belong to intrinsic (or recurrent) connections from the same area [46].

Robust BOLD responses can be observed in correlation with LFPs even in the absence of neural spiking [47]. Therefore, although LFPs and neural spiking are often correlated [47], it is possible that SN/VTA LFPs elicit a SN/VTA BOLD response while the firing of dopaminergic neurons remains unchanged or even decreases. To what extent such a scenario is physiologically plausible in the SN/VTA is unclear. To our knowledge, there are no publications that have compared SN/VTA LFPs and neural firing in rodents (A. Grace, personal communication) and non-human primates (see Ref. [48] for a relationship between VTA LFPs and prefrontal cortical up-states). So, it is unclear to what extent SN/VTA LFPs and dopaminergic firing are indeed correlated in response to motivationally salient events. However, it is interesting to note that in the SN/VTA the number of afferents and intrinsic connections is not as large as in the cortex (A. Grace, personal communication). This might suggest that, particularly in the SNc where ~70% of neurons are dopaminergic, dopamine neuron firing could be closely correlated with BOLD responses to afferent input.

In Figure 2, we summarize several plausible physiological mechanisms that might contribute to SN/VTA BOLD responses. Given the lack of decisive physiological evidence...
from animal studies, it is premature to pinpoint one particular mechanism from Figure 2 as driving SN/VTA BOLD responses. However, for novel or reward-related stimuli, the most parsimonious mechanism to elicit an SN/VTA BOLD response is afferent glutamatergic drive (from the prefrontal cortex [PFC] or mesopontine nuclei; Box 1) onto dopaminergic neurons (number 1 in Figure 2). How such BOLD responses spatially overlap with phasic dopaminergic responses will depend on whether glutamatergic inputs and the size of the tonically active population spatially overlap with burst firing [31] (number 3 in Figure 2; Box 2) and whether glutamatergic drive onto the silent population of dopamine neurons (i.e., neurons that are not tonically active because they are inhibited by γ-aminobutyric acid [GABAergic] projections from the ventral pallidum; number 2 in Figure 2) cause a BOLD signal. GABAergic projections from the ventral pallidum should decrease SN/VTA BOLD responses [49] (Box 2), whereas the contribution of GABAergic neurons might vary with presynaptic, direct or indirect inhibition.

**Convergence between the BOLD signal and dopamine release**

To what extent is it possible that the magnitude of the fMRI SN/VTA response to reward-related stimuli contains information about the magnitude of dopamine release? Several findings support the possibility of a quantitative relationship. Striatal levels of dopamine as measured with a premortem 6-[18F]fluorodopa PET were strictly proportional to postmortem counts of dopaminergic cell densities in the substantia nigra [50]. Pharmacological fMRI studies [2,51] suggested a quantitative role for dopaminergic signalling in the ventral striatal reward response. Furthermore, striatal BOLD responses evoked by amphetamine, a drug that enhances dopamine release, correlated with the number of dopamine neurons that survived a neurotoxic lesion to the SN/VTA in rhesus monkeys [52].

More recently, we observed that SN/VTA BOLD responses to reward-predicting stimuli were positively correlated with reward-related dopamine release in the nucleus accumbens (NAc) as indexed by [14C]raclopride PET [53]. Notably, we found a quantitative correlation between dopamine release and the compound SN/VTA BOLD response (see Ref. [54] for a discussion of dopamine effects on the BOLD response). This finding is compatible with the primate physiology that mesolimbic projection neurons and reward-related activations are not confined to the VTA but have expanded into the SNC.

Because age and disease might differentially affect dopaminergic subregions, considering structural integrity within the human SN/VTA seems relevant. Recently, this has been measured using volumetry [55,56], magnetization transfer ratio [13,57] and diffusion weighted imaging [13]. In older adults, the magnetization transfer ratio of the SN/VTA correlates with mesolimbic fMRI responses to novel stimuli [57] and cognitive performance in neuropsychological tests of learning and memory [13]. Hence, a tripartite quantitative relationship including structural magnetic resonance (MR) parameters of the SN/VTA, SN/VTA BOLD responses to rewarding or novel stimuli and striatal dopamine release with PET might be a feasible goal to achieve in the near future [58]. Furthermore, new structural imaging protocols for the high neuromelanin content of dopamine neurons (like noradrenergic neurons) could improve anatomical localizers for fMRI studies [59].

**Possible dissociations between fMRI responses and dopamine release**

With aversive stimuli or reductions or omissions of expected rewards, it is conceivable that BOLD responses and dopamine release might dissociate (Figure 2). For strong aversive stimuli (pain elicited by tail pinch or foot shock), most dopaminergic neurons in the rodent VTA show a suppression of neural firing during the duration of the aversive stimulus. Although the decrease in firing rate is subtle (from ~4 Hz to ~3 Hz, i.e., ~25%), this should be associated with either a subtle decrease or no change in dopamine release [60]. It is possible that the inhibition of dopaminergic firing is associated with increased afferent glutamatergic drive onto inhibitory interneurons in the SN/VTA and the related increase in LFP power. In such a scenario, there would be a dissociation of BOLD responses and dopamine firing and release. A recent study showed that there was no significant decrease of VTA BOLD (measured with an MR field strength of 3 Tesla) to mild aversive outcomes caused by an absence of reward or mild loss of money [21]. Note, however, that fMRI might be insensitive in detecting afferent drive to interneurons because only a portion (29% in the SNc and 35% in the VTA) of the neuronal population is GABAergic. Thus, a dissociation between increased BOLD and decreased dopamine activity might emerge only with very sensitive fMRI technology (e.g., at 7 Tesla). Also, as mentioned earlier, loss of reward might modulate dopamine neurons in the SNC and retrorubral field rather than the VTA. Other forms of dissociations between BOLD and neuronal measures are also conceivable (Box 2; habenular complex, number 8 in Figure 2).

**Separable SN/VTA circuits and functional dissociations**

Although the aforementioned analysis suggests little evidence for global anatomical and functional segregation across dopamine cell groups, more local segregation might occur within cell groups. Converging evidence suggests that SN/VTA dopaminergic neurons projecting to different targets are regulated by different afferents and, therefore, that subsets of SN/VTA dopamine neurons participate in separate circuits [61]. This is indicated by regionally dissociable effects of VTA stimulation with compounds specifically increasing dopamine levels in the PFC but not in the NAc [62] and others causing an increase in the NAc and a decrease in the PFC [63]. Dopamine neurons projecting to the PFC have higher baseline firing rates, fire more action potentials in bursts, have a higher turnover and metabolism of dopamine and might be more sensitive to mild stressful stimuli. These features might reflect the nature and content of PFC input to mesoprefrontal dopamine neurons [64]. PFC inputs to SN/VTA could cause activation of mesoprefrontal and inhibition (via GABAergic mechanisms) of mesolimbic dopamine neurons [64]. The implication of this [64] is that prefrontal hypofunction, for example in schizophrenia, would decrease dopamine input to PFC.
while causing a hyperdopaminergic state in mesolimbic structures [65].

In fMRI studies, this functional segregation could explain functional dissociations between SN/VTA novelty and reward BOLD responses [66]. For instance, novelty consistently activates the SN/VTA but not the NAc [57,59,67,68], whereas rewards and stimuli predicting rewards reliably activate both [19,21,66,69,70]. One possible explanation is that PFC inputs (which inhibit mesolimbic dopamine projections) are more pronounced for novelty than rewards.

**Future perspectives: high-resolution imaging of SN/VTA: gradients, connectivity and functional dissociations**

To measure the compound signal of the SN/VTA complex with its volume of ~350–400 mm$^3$ [55], fMRI voxel sizes of ~3 mm$^3$ isotropic seem to provide sufficient resolution, yielding 20–25 voxels from this region. However, to assess functional dissociations between the SN and VTA in humans, high-resolution fMRI (voxel sizes of 1.5 mm$^3$ and smaller) might be useful, particularly when combined with spatial priors [71] about the distribution of dopaminergic neurons and about gradients of connectivity. Combining high-resolution fMRI and PET is another promising avenue particularly if both modalities can be registered simultaneously and modelled together on a trial-by-trial basis. This approach would reveal to what extent regional SN/VTA BOLD responses lead to dopamine release in different target regions (given PET ligands that are sufficiently sensitive to detect dopamine release outside the striatum). Finally, multivariate pattern classification algorithms [72] can indicate whether spatially distinct neural response patterns in the SN/VTA can distinguish different types of motivationally salient events (e.g. rewards, novelty and aversive events).

To our knowledge, the pool of ‘silent’ dopamine neurons remains to be studied in the behaving primate and it is, therefore, difficult to estimate to what extent differences between silent and tonically active pools of dopamine neurons contribute to BOLD responses (Box 3). Dynamic causal modelling [73] might, in principle, provide an opportunity to model the slow effects of tonic SN/VTA modulation of projection areas such as the NAc and distinguish this from rapid phasic SN/VTA modulation. Such approaches could reveal whether tonic and phasic modes of SN/VTA activity are correlated in terms of the distribution and size of their projection targets and whether they are contextually coordinated in terms of their inputs and outputs.

**Conclusions**

This article aimed to highlight the species differences that should be considered when imaging the SN/VTA with the objective of making inferences about dopaminergic neurotransmission. The commonly used neuroanatomical and functional distinction between nigro-striatal (SNc to the dorsal striatum) and mesolimbic and mesocortical pathways (VTA to cortical and limbic structures) is not as clear cut in humans as many publications imply. Indeed, from an extracellular perspective, functional differences between primate VTA and SNc currently seem subtle. Moreover, mesolimbic and mesocortical dopaminergic projection systems are dispersed throughout the SN/VTA in humans (Figure 1). By focusing exclusively on human VTA, 85% of the total human dopamine neurons in SNc, retrorubral field and the human-specific midbrain reticular field can be missed. We suggest instead that in humans the compound signal from the entire dopaminergic region has most potential to inform about dopaminergic neurotransmission. A limitation of imaging studies to the VTA region has limited utility.

By contrast, high-resolution fMRI could prove useful in multidisciplinary, cross-species experiments in human and non-human primates (and in elucidating a role of the human-specific midbrain reticular field). Similar experimental paradigms using comparable stimuli and rewards in both species are feasible. One possible approach is to study the relationship between LFPs and tonic and phasic dopaminergic responses and their functional gradients using extracellular recordings in non-human primates. These data could then be related to functional response gradients and regional dopamine release in humans studied by combining PET and high-resolution fMRI (Box 3). To achieve this goal, rodent models of tonic and phasic firing modes of dopamine neurons need to be established in non-human primates. Through such multidisciplinary experiments, fMRI of the SN/VTA could become a useful tool to study dopaminergic neuromodulation in healthy and clinical populations.

**Acknowledgements**

This work was supported by a Wellcome Trust Project Grant (to E.D., 81259; www.wellcome.ac.uk), the Deutsche Forschungsgemeinschaft (Grant No. KFO 163; www.dfg.de), the German Helmholtz Association (virtual institute grant No. VH-VI-125; www.helmholtz.de), a Marie Curie Fellowship (to M.G.-M.; www.mariecurie.org.uk), the Swiss National Science Foundation (Grant No. KFO 163; www.snsf.ch) and the Roche Research Foundation (P.N.T.; www.research-foundation.org). We thank Karl Friston for helpful discussions and comments on the manuscript.

**References**


38 Haber, S.N. et al. (2000) Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J. Neurosci. 20, 2369–2382


53 Peters, Y. et al. (2004) Prefrontal cortical up states are synchronized with ventral tegmental area activity. Synapse 52, 143–152


76 Beckstead, R.M. et al. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.* 175, 191–217

77 Nair-Roberts, R.G. et al. (2008) Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience* 152, 1024–1031


84 Fudge, J.L. and Haber, S.N. (2001) Bed nucleus of the stria terminalis and extended amygdala inputs to dopamine subpopulations in primates. *Neuroscience* 104, 807–827


