Selective mesoaccumbal pathway inactivation affects motivation but not reinforcement-based learning in macaques.

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Summary

Rodent studies have demonstrated the role of the mesoaccumbal circuit in reinforcement-based learning. Importantly, however, while phasic activity of the ventral tegmental area (VTA) contributes to reinforcement learning, rodent evidence suggests that slow changes in tonic VTA activity and associated accumbal dopamine release help regulate motivational behavior. Nonetheless, the consequences of sustained blockage of the mesoaccumbal circuit for motivation and reinforcement learning have not yet been examined in primates. Using a double-infection viral vector technique, we demonstrate that selective, unidirectional and reversible blockage of the primarily dopaminergic mesoaccumbal circuit in monkeys increased network-level functional connectivity, especially in fronto-temporal cortex. These global network changes were not associated with deficits in reinforcement learning during an object discrimination reversal task. In contrast, sustained mesoaccumbal inactivation greatly reduced motivation for performing a motivation-based decision-making task. Thus, the mesoaccumbal pathway in primates is critical for high-effort motivation, but not for all forms of reinforcement-based learning.

Introduction

Although countless reward-related functions are attributed to dopaminergic (DA) midbrain neurons, our understanding of their exact roles in learning and motivational behavior in primates remains tentative. Phasic firing of dopamine cells in the VTA encodes reward prediction errors (RPEs), signaling the difference between expected and obtained rewards (Schultz et al., 1997), which is instrumental for learning (Fiorillo et al., 2008; Pessiglione et al., 2006; Schultz, 1998, 2007, 2015; Schultz et al., 1997; Wise and Rompre, 1989). Altering dopamine signaling either nonspecifically through

systemic drug injections, or in a site-, pathway- or cell-type specific manner affects reinforcement-based learning, demonstrating its dependence on dopamine in several species including humans (Cohen et al., 2012; Eshel et al., 2016; Frank et al., 2004; Haber and Knutson, 2010; Saunders et al., 2018; Stauffer et al., 2016; Steinberg et al., 2013). In rodents, distinct dopaminergic striatal pathways may underlie different aspects of reinforcement-based learning (Saunders et al., 2018; Saunders and Robinson, 2012; Steinberg et al., 2013; Taylor and Robbins, 1984), with the VTA projections to the nucleus accumbens (NAc) thought to mediate the acquisition of incentive value during such learning (Saunders et al., 2018). In addition, Yin et al. (2008) suggested that the NAc is necessary for the acquisition and expression of certain Pavlovian responses and contributes to the motivational control of instrumental performance, whereas dopaminergic projections to the dorsal striatum are necessary for the acquisition and expression of goal-directed actions. Other studies, however, have challenged the idea that reinforcement learning is strictly dopamine dependent (Shiner et al., 2012; Yin et al., 2008). Strong counterarguments come from experiments using mice lacking tyrosine hydroxylase, hence being completely dopamine depleted. Such mice exhibit reinforcement-based learning abilities but lack any motivation to engage in goal-directed behaviors, which can be triggered by giving L-Dopa (Palmiter, 2008). In fact, a potential caveat in many studies is that learning and performance or motivation-related effects cannot easily be dissociated, as these concepts are highly interrelated. Finally, most of the cell-type-specific or region-specific causal studies of dopaminergic-dependent reinforcement learning are restricted to rodents, while human studies using systemic injections of (ant)agonists may induce potentially confounding off-target effects. Hence, current evidence about the specific pathways involved in

dopaminergic-dependent reinforcement-based learning in primates is circumstantial at best.

Equally importantly, dopamine levels may also depend heavily on the ongoing behavior itself, without necessarily considering past experiences or affecting future behavior. This is consistent with a pure reward-prediction or value signal driving instant motivation rather than a learning signal per se (Bromberg-Martin et al., 2010; Hamid et al., 2016; Salamone and Correa, 2012). Interestingly, reduced motivation might also partially explain motor deficits typically attributed to a malfunctional dopaminergic system. Parkinson's patients, whose dopaminergic neurons in the VTA have degenerated, may be less motivated rather than unable to move (Berke, 2018; Mazzoni et al., 2007). Both tonic VTA activity, and local presynaptic mechanisms in the NAc independent of spiking activity of DA neurons (Mohebi et al., 2019) which control dopamine release, have been implicated in the regulation of motivational behavior in rodents (Berridge, 2007; Berridge and Kringelbach, 2008; Salamone et al., 2007). Although the dopaminergic projections from the VTA to the NAc are deemed instrumental in motivational behavior (Kalivas and Volkow, 2005; Wise, 2006), the latter assertion remains controversial (Palmiter, 2008), even for rodents.

Hence, it remains an open question whether mesoaccumbal signaling in primates can be causally implicated in both motivational and reinforcement-based learning. Therefore, using a reinforcement-dependent object reversal discrimination task, we tested in macaques whether the connections running specifically from VTA to NAc promote learning, and whether this circuit drives effortful behavior in a motivation task. To achieve these goals, we reversibly blocked the connections between the VTA and NAc, which are primarily dopaminergic, using a double infection technique in which injection of a retrograde vector into the projection area of the target cells is combined

with injection of an anterograde vector at the location of their somas (Kinoshita et al., 2012). We then tested the effect of this pathway-selective blockage separately on a reinforcement learning and a motivation task. The former task was a variant of an object reversal discrimination task (Chau et al., 2015) in which the monkey had to select one of two stimuli with different, but randomly changing, reward probabilities. In the motivation task, the monkey also had to select one of two stimuli randomly drawn from a set of five, which were linked to different reward sizes and motivation levels needed to obtain them. Histology showed reliable targeting of dopaminergic connections between the VTA and NAc in rhesus monkeys, while resting state fMRI demonstrated that blocking the VTA-NAc pathway significantly altered functional connectivity patterns. Moreover, we show for the first time that the mesoaccumbal dopamine pathway in primates is necessary for motivating high effort behavior, but not for all forms of reinforcement-based learning.

Results

Selective, unidirectional and reversible inactivation of the VTA-NAc pathway in macaques

A double infection technique (Kinoshita et al., 2012) was used to selectively and reversibly block the primate mesoaccumbal pathway (i.e. the connections from VTA to NAc). To achieve this, we first injected the NAc with an efficient retrograde gene transfer vector (either HiRet or NeuRet, Supplemental Table 1) coding for an enhanced GFP-tagged (EGFP) tetanus neurotoxin (eTeNT) under the control of a tetracycline-responsive element (TRE) (vector 1, Figure 1A, see STAR methods). Next, an adeno-associated viral vector (AAV2) carrying a cytomegalovirus (CMV) promoter and

efficient reverse tetracycline transactivator (rtTAV16) gene was injected into the VTA (vector 2, Figure 1A; see also TH stained section showing the VTA and subdivisions of the substantia nigra in Figure 1D). After a waiting period (4 - 9.5 weeks), resting-state fMRI (rsfMRI) and behavioral experiments began (Figure 1B, see detailed timeline of the experiment in STAR Methods). During these experiments we alternated between periods with oral doxycycline administration (dox-on), and periods without (dox-off). When doxycycline is administered to the transduced animals, doxycycline binds to the rtTAV16 and activates the TRE promoter triggering the expression of the eTeNT and EGFP. This neurotoxin then selectively suppresses synaptic transmission in doubly-infected neurons (Kinoshita et al., 2012) (Figure 1A, C), and EGFP labels those projection neurons that were effectively suppressed.

To examine whether doubly-infected neurons were restricted to the mesoaccumbal pathway, immunohistochemistry (IHC) was used to locate fluorescent protein (FP) expression within transduced cell bodies and axonal trajectories. FP positive neurons (either EGFP+ or mCherry+, see supplemental Table 1) were found to be largely restricted to the VTA and its border with the substantia nigra (in control monkey M43 without eTeNT expression, an estimated 1500 mCherry+ neurons, and 650 EGFP+ neurons) confirming the selective transduction of the mesoaccumbal pathway (Figure 1E and 2, Figure S1 and Supplemental Movie S1). The IHC results of M43 are confirmed in experimental subjects M36 and M30, indicative for double-infected neurons that also produced eTeNT (Figure 2 and Figure S2). To assess the transduction capacity of both vectors, in situ hybridization (ISH) against GFP (Hi-Ret/NeuRet) and rtTAV16 (AAV2) was performed (Figure S2C-F). ISH revealed a high proportion of transduced neurons at the VTA injection site. The larger number of infected VTA neurons found with ISH compared to IHC (Figure S2A, C, D, F) suggests

that the number of cells with functional levels of eTeNT is underestimated by IHC.

Because the mesoaccumbal circuit provides the major dopaminergic input to the NAc (Cox and Witten, 2019), we hypothesized that most doubly-infected mesoaccumbal neurons would also be dopaminergic. A double-staining procedure revealed that, qualitatively, the largest fraction of FP+ neurons (~80%) co-expressed tyrosine hydroxylase (TH), confirming that doubly-infected cells were primarily dopaminergic (Figure 1C). It should be noted that the neurons in the other 20% were either non-dopaminergic in nature (e.g. GABAergic or glutamatergic), or dopaminergic neurons in which the FP levels were too low to be detected with IHC (i.e. false negative detection of DA). Finally, the spread of mesoaccumbal collaterals was also examined, since VTA neurons may exhibit multibranched axons targeting multiple regions, at least in rodents (Aransay et al., 2015). To examine the topography of these collaterals, we stained the NAc and surrounding areas for GFP or mCherry (Figure 3). FP+ fibers were found in the NAc and surrounding structures (caudate, internal capsule, putamen and ventral pallidum) but with little labeling in the cortex indicating that mesoaccumbal collaterals were relatively restricted to the ventral half of the basal ganglia. Therefore, the primate VTA to NAc circuit is not an isolated pathway, as it is commonly described, but a more diffuse route that shares information, primarily with adjacent ventral regions of the basal ganglia.

Pathway selective VTA-NAc inactivation increases mesolimbic functional connectivity.

To assess the effect of selective inactivation of the mesoaccumbal pathway on functional connectivity, rsfMRI (Mantini et al., 2013; Vanduffel et al., 2001) was performed in animals in which the VTA-NAc pathway was uni- or bilaterally blocked (to

which we refer as uni- or bilateral eTeNT animals, respectively), as well as in control animals (see STAR methods). Correlations of the time series between all possible combinations of ROIs were computed and r-to-z transformed, thereby correcting for the degrees of freedom for the autocorrelation in the time series (see STAR Methods).

Surprisingly, after VTA-NAc inactivation, we observed significantly increased functional connectivity between the VTA and NAc of bilateral eTeNT monkeys (dox-on vs. dox-off; Wilcoxon rank-sum test: M36, $p = 0.0025$; M40, $p = 0.0044$; group, $p = 2.680 \times 10^{-4}$) while no significant effect was found for the control animal (M43, $p = 0.0542$). Consistent with this, the unilateral eTeNT monkey (M30) displayed increased mesoaccumbal functional connectivity in the injected hemisphere (Wilcoxon rank-sum test: $p = 1.298 \times 10^{-5}$) and decreased functional connectivity in the control hemisphere (Wilcoxon rank-sum test: $p = 0.0015$). Importantly, functional connectivity reflects correlated activity but not anatomical connectivity. Therefore, this data indicates that correlated activity between the VTA and NAc increases after blockage of the unidirectional VTA-NAc connections.

We next examined the effect of mesoaccumbal blockage on the functional connectivity of VTA and NAc with the rest of the brain using a seed-based ROI approach (Figure 4A, Figure S3A-B). Increased functional connectivity was observed between VTA and several brain regions including the cerebellum, early visual cortex, anterior inferotemporal cortex, SII, hippocampus, orbital frontal cortex, thalamus, midbrain. Only a few regions showed decreased functional connectivity with VTA (inferotemporal cortex, hippocampus, basal ganglia). Likewise, functional connectivity between the NAc and several brain regions was found to be significantly elevated after doxycycline administration (Figure 4A, and S3A: orbital frontal cortex, putamen, temporal cortex, visual cortex and cerebellum). Overall the seed-based analysis

confirmed that inactivation of the mesocaccumbal circuit predominantly increased functional connectivity between mesoaccumbal nodes and the rest of the brain.

To quantify the effect of dox administration outside the mesoaccumbal circuit, a brain-wide region-of-interest (ROI) analysis was performed (Figure 5A-D; Figure S4; Supplemental Table 2). We found that interregional functional connectivity in bilateral eTeNT animals was more broadly modulated by doxycycline administration compared to control animals (FDR correction, $p < 0.05$; Figure 5A-B, Figure S4A-B). In addition, interregional functional connectivity increased predominantly in bilateral eTeNT animals (Figure 5C; see Figure 6 for a cortical representation of the number of increased and decreased functional connections per area). In the unilateral eTeNT animal, increased functional connectivity was observed predominantly within the targeted hemisphere (Figure 5D).

The most informative changes in functional connectivity, however, are most likely those which are correlated with the change in functional connectivity between the VTA and NAc. Therefore, we performed an additional analysis whereby we correlated the functional connectivity change between the VTA and NAc with the significantly modulated functional connections across all other ROI pairs. Connections showing significantly higher correlations with functional connectivity change between the VTA and NAc during dox-on than dox-off are shown in Figure 4B-D and Figure S5. Corroborating the results of our previous analysis showing more dox-induced increases than decreases in functional connectivity, there were also more pairs showing increased functional connectivity that correlated positively with the change in functional connectivity between the VTA and NAc (hot color code), as opposed to pairs that show decreased functional connectivity (cold color code). Correlated functional connections involved regions of the anterior temporal pole (within the STS and lateral

fissure), orbitofrontal cortex, vLPFC and dLPFC, as well as premotor and posterior cingulate cortex which were also most strongly affected by the inactivation of the mesolimbic pathway. Negative functional connectivity changes were restricted to the anterior bank of the rostral portion of the anterior bank of the STS and entorhinal cortex.

Reversible blockage of the VTA-NAc pathway reduces motivated behavior

After confirming the surprisingly widespread physiological effects of sustained mesoaccumbal blockage, we examined this circuit's role in regulating motivated behavior using a motivation-based decision-making task. This task required animals to choose between different visual cues each of which were associated with a distinct effort level (fixation time required to complete a trial) and reward size (volume of juice delivered). Individual trials began with a fixation period (1s), followed by the presentation of 2 cues which were randomly selected from the set of five cues, and which appeared at 2 of the 8 possible positions (Figure 7A). After cue-presentation, animals selected a cue using a saccade. Then the animals had to maintain fixation on that cue for the duration of time associated with the selected cue. If fixation behavior was maintained for this duration, the trial was considered correct and a juice reward was delivered. Trials in which the monkey selected a target but did not maintain fixation were deemed uncompleted. Longer fixation times reflected high-motivation behavior and were associated with larger rewards (Figure 7, Figure S6).

We hypothesized that if activity in the mesoaccumbal pathway regulates motivation to select high-effort cues, sustained inactivation of this circuit should reduce the animal's preference for higher-effort cues. To test this, we measured behavior during a series of daily sessions of the motivation-based decision-making task with and without doxycycline administration. One full cycle of dox-off and dox-on sessions

was considered a complete round of the experiment. Because of the reversible nature of the double infection technique (Kinoshita et al., 2012), each animal performed multiple rounds to detect reproducible behavioral changes.

To assess how preference for cues changed after doxycycline administration, a cue preference index $\left(\frac{\text{\# of cue X selections}}{\text{\# of cue selections}} \right)$ was computed for all correct trials. Examination of cue preference during example rounds demonstrates that, during the later sessions of the dox-on period, bilateral eTeNT animals exhibited a reduced preference for high-effort cues while the preference for low-effort cues increased (Figure 7D). To quantify this change in behavior, we compared the cue preference index during the later sessions (last third) of the dox-off and dox-on periods (see STAR Methods). This analysis confirmed the aforementioned example data: for bilateral eTeNT animals, doxycycline administration significantly decreased preference for the highest effort cues (D & E) and increased preference for the lowest effort cues (A & B), confirming that sustained mesoaccumbal blockage reduced the preference for high-effort behaviors (Figure 7E, Supplemental Table 3). The same shift in cue preference was found when both correct and uncompleted trials were included (Figure S6B), indicating that changes in cue selection, and not trial completion, drove the observed differences in cue preference. This was also reflected in a decreased preference for selecting the higher-effort cue that was found for bilateral eTeNT animals during doxycycline administration (Figure 7F).

We next tested whether mesoaccumbal pathway blockage affected the motivation to complete trials. We found that the proportion of uncompleted trials increased significantly for bilateral eTeNT monkeys (Figure 7G). Moreover, the frequency of uncompleted trials was significantly increased for the higher-effort trials

(Figure S6C), suggesting that mesoaccumbal blockage decreased motivation for completing high-effort tasks. Importantly however, the times at which cue fixation was interrupted in the uncompleted trials were the same for dox-off and dox-on periods (Figure S6D), indicating that there were no differences in the animal's capacity to fixate. In addition, reaction times were not affected by doxycycline administration (Figure S6E, right panel), providing further evidence that doxycycline did not affect oculomotor control. To test if mesoaccumbal blockage affected motivated behavior more generally, we compared the time extents that the monkeys worked during daily dox-on and dox-off sessions. Note that the animals were allowed to continue a session until they stopped performing the task. We cannot precisely estimate how long after discontinuing the dox treatment the monkey's motivation remained compromised because each dox-on cycle was followed by a rest period of 9 to 27 days during which the monkeys were not tested. In the beginning of the following dox-off session, the behavior was normalized (see e.g. Figure 7D). Inactivation of the mesoaccumbal pathway did not alter the duration that animals worked (Figure S6E, left panel), suggesting that mesoaccumbal inactivation selectively affected motivation for high effort behaviors while preserving more general motivational behavior requiring low effort.

Reversible blockage of the VTA-NAc pathway does not affect reinforcement learning

While rodent studies using phasic activation of the mesoaccumbal circuit have directly implicated this pathway in reinforcement learning, manipulations of dopamine signaling in NAc provide indirect information that tonic signaling (Salamone and Correa, 2012), or DA release triggered by local processes (Mohebi et al., 2019), preferentially affects

motivation rather than reinforcement learning in rodents. To directly test the effect of long-term blockage of mesoaccumbal signaling on reinforcement learning in primates, bilateral eTeNT and control monkeys performed an object discrimination reversal task, that relied on reinforcement learning, during cycles of on and off doxycycline administration.

During each run (900 trials) of this task, monkeys had to select between two visual cues, each associated with a distinct reward probability. The initial cue-reward probabilities (cue A = 1/3 rewarded trials, cue B = 2/3 rewarded trials) were reversed twice at randomized time points during each run. To optimize the amount of reward received during a run, the monkeys needed to use information from previous trials to select the cue associated with the higher probability of reward. Monkeys initiated trials of the task by maintaining fixation for a randomized period (1 - 1.5s) on a central point, after which two visual cues were simultaneously presented in the periphery (Figure 8A-B). Cue selection was accomplished by a saccade onto one of the cues. Juice reward administration depended on the reward probability associated with the selected cue.

To assess the effect of doxycycline administration, we aligned cue preference data to the cue-reward probability reversals and fit a sigmoid function (Figure 8C). No significant difference was found in any of the sigmoid function parameters between dox-off and dox-on periods for both the bilateral eTeNT and control animals (Supplemental Table 3). To further analyze the effect of doxycycline administration on trial-by-trial choice behavior, we modeled bilateral eTeNT and control group performance using an indirect actor model (Figure 8D, Supplemental Table 3). Again, no difference in the learning rate, exploration parameter, or fit of the model was found between the dox-off and -on periods for either group. This data suggests that, with this paradigm, sustained blockage of the mesoaccumbal pathway did not significantly

affect reinforcement learning. As in the previous task no significant differences in the reaction time or overall working time were observed (Figure S7).

Discussion

We used a double-infection viral vector technique to specifically block the connections between the VTA and NAc. Although pathway-selective interference methods are commonplace in rodent research (Tye and Deisseroth, 2012), such tools have been used far less frequently in monkey studies (Inoue et al., 2015; Kinoshita et al., 2019; Kinoshita et al., 2012; Nurminen et al., 2018; Tohyama et al., 2017). Here, we adapted the protocol to distinguish connections between two subcortical structures. Histology revealed successful targeting of mainly dopaminergic projection neurons between VTA and NAc. Although efficiency was rather low (an estimated ~700 to 1500 neurons per VTA were doubly-infected), a high proportion (~80%) of transduced neurons were dopaminergic, indicating that we had successfully targeted primarily dopaminergic neurons of the VTA-NAc circuit. Importantly, we achieved pathway-selectivity since only the double-transfected cells are capable of expressing eTeNT, thereby inhibiting neurotransmitter release at their synaptic terminals in the NAc. Despite rather low transduction rates, however, we found surprisingly large effects on network functional connectivity and behavior as discussed in detail below. Possibly our FP+ neuron count is an underestimation of all doubly-infected neurons, insofar as ISH suggested much higher proportions of transduced neurons at the VTA injection site compared to IHC. These findings are in line with suggestions that, relative to functional gene expression, the number of FP copies required for detection using IHC needs to be higher (K. Inoue and M. Takada, personal communication). Alternatively, the loss of relatively small

345 numbers of mesoaccumbal projection neurons may disproportionally impact network
346 functions and behavior in primates.

347 The sustained decrease of signal propagation between the VTA and NAc
348 revealed an increase in functional connectivity, as measured with fMRI, within the
349 mesolimbic circuitry and far beyond. The most prominent changes, that also correlated
350 with the VTA-NAc changes in functional connectivity, were observed in anterior
351 temporal, orbitofrontal and dorso- and lateral prefrontal cortex (Figure 4), regions
352 implicated in reward and motivational processing and receiving strong dopaminergic
353 input from the ventral midbrain (Fields et al., 2007; Williams and Goldman-Rakic,
354 1998). While seemingly counterintuitive, mesoaccumbal blockage may increase
355 functional connectivity by reducing the inhibitory output of the predominantly
356 GABAergic NAc neurons, which also provide a feedback signal to regulate the
357 dopaminergic output of the VTA (Day and Carelli, 2007; Robison and Nestler, 2011;
358 Scofield et al., 2016). In turn, this release of feedback-inhibition in the VTA itself may
359 increase the functional coupling with the latter structure through its extra-accumbal
360 projection sites. In addition, the NAc itself couples via GABAergic medium-spiny
361 neurons (MSNs) with multiple targets (Cox and Witten, 2019), whereby tonic and
362 phasic DA activity affects mainly D2 (indirect pathway) and D1 receptors (direct
363 pathway), respectively (Palmiter, 2008). Hence, diminished dopamine release in NAc
364 may reduce the influence of the MSNs on target regions and evoke a cascade of
365 elevated functional connectivity, exactly as we observed (Salgado and Kaplitt, 2015).
366 Furthermore, our findings are also consistent with a human rsfMRI study
367 demonstrating that increases in dopaminergic signaling using systemically
368 administered methylphenidate resulted in reduced mesolimbic functional connectivity
369 (Ramaekers et al., 2013), and with DA-dependent functional connectivity changes

observed in human orbitofrontal cortex (Kahnt and Tobler, 2017). Also consistent with our study, early- and late-stage Parkinson patients show increased functional connectivity compared to healthy controls, which is attenuated using dopaminergic treatments (Silberstein et al., 2005; Stoffers et al., 2008). In summary, our data suggest that mesoaccumbal signals may act as major attenuators of functional connectivity across many brain regions thereby regulating information flow within these systems in a manner relevant for goal-directed behavior. Intriguingly, profound increases in functional connectivity are associated with decreased behavioral outputs in a motivational task.

A major result of the present study is the dissociation between effects of mesoaccumbal blockage on motivation and reinforcement-based learning. Despite reported evidence that changes in dopamine affect learning in an RPE-dependent manner (Cohen et al., 2012; Eshel et al., 2016; Frank et al., 2007; Frank et al., 2004; Saunders et al., 2018; Steinberg and Janak, 2013), the distinction between learning and task performance in such studies is not always straightforward (Shiner et al., 2012). Although our results are in line with literature asserting that reinforcement learning does not critically depend on DA (Shiner et al., 2012; Yin et al., 2008), alternative interpretations may exist. First, the most refined causal evidence supporting a positive relationship between DA and reinforcement learning derives from rodent studies, which is somewhat corroborated by far coarser causal primate data (Arsenault et al., 2014; Arsenault and Vanduffel, 2019; Frank et al., 2007; Frank et al., 2004; Pessiglione et al., 2006; Stauffer et al., 2016). In addition, it should be noted that important species differences between rodents and primates have been documented regarding dopamine receptor distributions and connection patterns (Berger et al., 1991). Thus, although we consider it unlikely, significant species differences in

dopamine-based reinforcement learning may exist between primates and rodents. Second, different dopaminergic pathways are likely involved in different types of reinforcement-based learning (Bromberg-Martin et al., 2010; Saunders et al., 2018; Saunders and Robinson, 2012; Taylor and Robbins, 1984). Here, we modified only one specific pathway and looked at one type of reinforcement-based learning (i.e., a variant of an object discrimination reversal task (Chau et al., 2015)). Thus, although NAc dopamine has been explicitly linked to reinforcement-based learning in rodents (Haber and Knutson, 2010), our specific reinforcement-based learning task may rely on other dopaminergic pathways originating in the VTA. The most parsimonious explanation of the present data, however, is that dopamine is not strictly required in all types of reinforcement-based learning, which is also consistent with pharmacological studies in humans (Shiner et al., 2012) and dopamine-depleted mice (Palmiter, 2008).

Our double-infection procedure may affect both phasic and tonic dopamine signaling within the VTA-NAc pathway. However, given the relatively slow timecourse of the mesoaccumbal inactivation effects on motivational behavior (Figure 7D), it may be argued that the observed effects are more deeply associated with sustained (tonic) reductions in dopaminergic release within the NAc. This is also consistent with original proposals that low tonic dopaminergic firing rates power motivational drive (Berke, 2018; Grace et al., 2007; Palmiter, 2008; Shohamy and Adcock, 2010). Several studies, however, have provided causal evidence that interruption of mesolimbic DA transmission does not affect motivational responses (Berridge, 2007; Berridge and Kringelbach, 2008; Salamone, 2007). Injection of dopamine antagonists, for example, does not affect primary motivation, although subjects tend to select a minimum-effort strategy for obtaining rewards (Salamone et al., 2007), just as in the present experiments with rhesus monkeys.

As with the dopamine pathways involved in reinforcement learning, there is also considerable controversy concerning the exact pathways underlying motivational behavior. Palmiter, et al. (2008) provided compelling evidence that the dopaminergic pathway between the substantia nigra pars compact (SNc) and dorsal striatum is particularly critical for motivation toward goal-directed behavior in mice, as opposed to the purported role of the ventral VTA-NAc pathway (Kalivas and Volkow, 2005; Saunders et al., 2018; Wise, 2006). Equally relevant is a recent study showing that dopamine release in target areas of the ventral midbrain, including the NAc, may be controlled by local synaptic mechanisms and not by (tonic) firing patterns of the dopamine cells themselves (Mohebi et al., 2019). Since the eTeNT employed in the present experiments blocks release of neurotransmitters presynaptically, it is to be expected that such local release mechanisms, driven by unaffected non-dopaminergic terminals in the accumbens, are also rendered inefficient. Irrespective of the exact release mechanisms in the NAc, our data show a clear involvement of the mesoaccumbal axons in motivational behavior.

In summary, sustained and selective reversible blockage of the dopaminergic projections between the VTA and NAc in rhesus monkeys leads to increased functional connectivity, as measured with resting-state fMRI within the mesolimbic circuitry, but also across many brain regions within the affected hemispheres. Despite the increased functional connectivity, mesoaccumbal blockage reduced the monkey's motivation to acquire larger rewards requiring more effort, yet reinforcement-based learning in an object reversal discrimination task was not compromised. Thus, sustained inactivation of specific connections between the VTA and NAc strongly affect global network functions and motivational behavior, but not all types of reinforcement-based learning in nonhuman primates.

Limitations of the study

Inherent to invasive nonhuman primate studies and because of ethical reasons, the number of subjects is limited. This strategy is also recommended by the 3R Guidelines. Therefore, typical studies with nonhuman primates rely on data of 2-3 subjects. Because of the complexity of the present study, combining MR-guided viral vector injections, quantitative histology, awake fMRI and cognitive paradigms, the number of animals in this study is slightly higher than usual. This is also the reason that multiple animals are used with slightly different experimental combinations. As a result, we do not make any inferences at population level, yet all reported findings are reproduced in the same animals and across at least two subjects (except for the unilaterally injected monkey, which was the first one tested).

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Declaration of Interests

The authors declare no competing interests.

Figure Titles and Legends

Figure 1. Pathway-specific and reversible blockage of VTA-NAc synaptic transmission. (A) Schematic diagrams of vector injections, doxycycline-induced gene expression and viral vector design. Doxycycline-controlled transcriptional activation mechanism: 1) Expression of a variant of reverse tetracycline transactivator (rtTAV16, Tet-on sequence); 2) Binding of rtTAV16 to the tetracycline responsive element (TRE) exclusively in the presence of a tetracycline derivative like doxycycline (dox) and thereby activating transcription; 3) Expression of the fluorescent protein (FP: GFP or mCherry) and enhanced tetanus neurotoxin light chain protein (eTeNT) downstream of the TRE promoter; 4) eTeNT cleaves the vesicle-associated membrane protein (VAMP) isoform 2 and inhibits enzyme secretion. **(B)** Experimental schedule (for details see STAR Methods). **(C)** Immunohistochemistry for dopaminergic (tyrosine

hydroxylase, TH; labeled in green) and mCherry (labeled in red) positive cells (left panel) in the VTA. Arrows indicate examples of double-labeled mCherry and TH positive VTA cells. **(D)** Localization of VTA and adjacent areas in a 40 μ m coronal section. DA neurons were visualized by anti-tyrosine hydroxylase IHC with AF594. **(E)** anti-GFP (left, green; FuG-B2-TRE-EGFP was injected in the left hemisphere) and anti-RFP (right, red; FuG-B2-TRE-mCherry was injected in the right hemisphere) positive- stained neurons from M43 across multiple VTA sections, but projected to one section. Scale bars are 50 μ m (C), 2 mm (D), and 10 mm (E). Abbreviations: AAV (adeno-associated virus), CMV (cytomegalovirus), cPPT (central polypurine tract), Hi-Ret (highly efficient retrograde gene transfer lentivirus), ITR (inverted terminal repeat), LH (left hemisphere), LTR (long terminal repeat), NAc (nucleus accumbens), Neu-Ret (neuron-specific highly efficient retrograde gene transfer lentivirus), PEST (peptide sequence rich in proline, glutamic acid, serine, and threonine), psi (p-element somatic inhibitor), RH (right hemisphere), RRE (rev response element), rsfMRI (resting-state functional magnetic resonance imaging), SV40pA (SV40 transcriptional terminator), VTA (ventral tegmental area) and WPRE (woodchuck hepatitis virus posttranscriptional regulatory element). See also Figure 2-3 and Figure S1-2 and Table S1.

Figure 2. Localization and spread of GFP-positive neurons in M36. Schematic of immunohistochemically stained green fluorescent protein (GFP) positive cells (green dots) located at the ventral tegmental area of the bilaterally injected monkey M36. GFP+ cells for four 40 μ m sections, separated by 80 μ m, were superposed onto the first representative section.

Figure 3. FP+ Axon tracking to ventral striatum (M43). **(A)** FP immunohistochemistry and neutral red counterstaining for the localization of mCherry positive axon and fiber termini in NAc. **(B)** mCherry positive axon tracing of section displayed in panel a. Small black boxes correspond to the inset shown in panel c (upper) and d (bottom). **(C)** Example inset with anti-RFP (mCherry) positive axon fibers and termini stained in black. **(D)** Example inset of anti-RFP+ fibers in caudate. Scale bars correspond to 0.1 mm (panel a-b) and 50 μ m in panel c and d. Abbreviations: Cd (caudate nucleus), ic (internal capsule), NAc (nucleus accumbens) and Pu (putamen).

Figure 4. Resting-state functional connectivity. **(A)** Doxycycline-induced changes in functional connectivity of the NAc (M36 and M40, bilateral eTeNT, dox-on vs. dox-off, t-scores, FDR corrected $p < 0.001$). **(B)** Plot of pairwise changes in functional connectivity which were correlated with the functional connectivity change between the VTA and NAc (FDR corrected $p < 0.05$). Pairs with increased or decreased functional connectivity during dox-on vs. dox-off are indicated in hot or cold colors, respectively (t-scores). **(C)** Back projection of the data in **B** on inflated representations of the cortex (lateral, ventral and medial views, and indication of subcortical areas from left to right, top to bottom). We counted the number of connections from any given ROI that were significantly more correlated with the change in functional connectivity of the VTA-NAc pathway with vs. without dox. Hot color code indicates the number of correlated functional connections showing increased functional connectivity during dox-on vs. dox-off. **(D)** Same as in **C** but functional connectivity decreased between dox-on vs. dox-off. Abbreviations: 12 (prefrontal area 12), 13 (prefrontal area 13), AL (anterior lateral, belt of the auditory cortex), as (arcuate sulcus), cf (calcarine fissure), cis (cingulate sulcus), cla (claustrum), cs (central sulcus), dox (doxycycline), EO

(entorhinal cortex, olfactory division), ER (entorhinal cortex, rostral division), F5 (agranular frontal area F5 (6Va/6Vb)), FDR (false discovery rate), fos (fronto-orbital sulcus), GP (globus pallidus), Hy (hypothalamus), ic (internal capsule), ios (inferior occipital sulcus), ips (intraparietal sulcus), lai (intermediate agranular insula area), lai (lateral agranular insula area), LH (left hemisphere), lu (lunate sulcus), NBM (nucleus basalis of Meynert), NLOT (nucleus of the lateral olfactory tract), ots (occipitotemporal sulcus), PACo Pir (periamygdaloid cortex o/piriform cortex), pos (parieto-occipital sulcus), PrCO (precentral opercular area), ps (principal sulcus), Pu (putamen), R (rostral, core region of the auditory cortex), RH (right hemisphere), RM (rostromedial, belt region of the auditory cortex), RPB (rostral parabelt region of the auditory cortex), RTp (rostrotemporal, polar part), sf (sylvian fissure), SII (secondary somatosensory area), STGr (rostral superior temporal gyrus), sts (superior temporal sulcus), TGA (agranular part of the temporal pole) and TGdd (dysgranular part of the dorsal temporal pole). See also Figure S3-5, STAR methods, and Table S2 for all abbreviations.

Figure 5. Changes in resting-state functional connectivity. Mean number of significantly increased **(A)**, decreased **(B)**, increased vs. decreased **(C)**, and ipsilateral_(increased vs. decreased) vs. contralateral_(increased vs. decreased) **(D)** ROI pairs calculated with bootstrapping (n=10,000). Error bars denote bootstrapped 95% confidence intervals. Asterisk denotes a significant difference from zero ($p < 0.05$). See also STAR methods.

Figure 6. Spatial overview of pairwise dox induced changes in functional connectivity. Spatial overview of ROIs showing increased (red color code) and decreased functional connectivity (cold color code) on inflated cortical views (lateral,

ventral, medial, and an indication of subcortical areas). Data correspond to the data in Figure S4. The respective color codes indicate the number of connections showing a significant increase or decrease in functional connectivity induced by dox for each ROI (FDR corrected for multiple comparisons at $p < 0.05$). Abbreviations: as (arcuate sulcus), cf (calcarine fissure), cis (cingulate sulcus), cs (central sulcus), fos (fronto-orbital sulcus), ios (inferior occipital sulcus), ps (principal sulcus), ips (intraparietal sulcus), lu (lunate sulcus), ots (occipitotemporal sulcus), pos (parieto-occipital sulcus), sf (sylvian fissure), sts (superior temporal sulcus). See also Table S2 for all abbreviations and STAR methods.

Figure 7. Motivation-based decision making. (A) Timing, cue positions and **(B)** eye position schematic of an example trial. Solid black line denotes eye position during cue A (option I) or cue D (option II) selection. **(C)** Example relationship of fixation duration and reward size for each cue (M40). **(D)** Cue preference index (PI, i.e. the strength of the preference for a particular cue) during an example doxycycline cycle for bilateral eTeNT (M40, M47) and control (M35) monkeys. **(E)** Mean normalized cue preference, **(F)** Percent selections of high-effort cue (i.e. percentage of all completed trials in which the monkey selects the cue, from the two cue options, with the highest reward and effort) and **(G)** Percent uncompleted trials (i.e. percentage of all trials in which the monkey aborts cue fixation before trial completion) during all doxycycline cycles. Data taken from last trimester of dox-off and dox-on periods. Error bar denotes SEM across days. Wilcoxon rank-sum test used to compute significance (* denotes $p < 0.05$). Results in panel D-F displays only data gathered from the correct (i.e. completed) trials. See also Figures S6 and Table S3, and STAR methods.

Figure 8. Reinforcement learning. (A) Example trial of reinforcement learning paradigm. **(B)** Schematic cue-reward probabilities reversals (2 per run) during an example run (900 trials). **(C)** Mean cue preference index aligned to cue-reward probability reversals (circles) during last third of dox-off (black) and on (orange) sessions. Lines denote mean sigmoid fit after bootstrapping analysis (10,000 iterations). **(D)** Learning rate (top panel) and exploration parameter (bottom panel) estimates from indirect actor models of the bilateral eTeNT (M36 and M40, black lines) and control (M24 and 34, blue lines) monkeys. Data from last third of dox-off and dox-on periods. Error bars denote 95% confidence interval as determined by bootstrapping analysis (10,000 iterations) (M30, $n = 17/13$; M36, $n = 18/22$; M40, $n = 39/39$; M24, $n = 30/19$; M34, $n = 13/15$ (runs Off/On). See also Figure S7, and STAR methods.

STAR Methods

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Resource Availability:

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Materials Availability: Histological data can be consulted upon request in the laboratory of Tadashi Isa. Department of Neuroscience, Graduate School of Medicine, Kyoto University, Kyoto, 606-8501, Japan.

Data and Code availability:

All numerical data presented in the figures are also available in tables (Supplemental Information, Data S1), and, if not, code is available on https://gitlab.com/u0100771/vancraeyenest_arsenault_supplementary.

Subjects

Eight rhesus monkeys (*Macaca mulatta*; M24, M30, M34-36, M40, M43 and M47) participated in this study (Supplemental Table 1). Monkeys M30, M36, M40 and M47 were injected with two viral constructs to induce pathway-selective blockade of synaptic transmission with enhanced tetanus neurotoxin (eTeNT). M30 was injected unilaterally (left hemisphere) while M36, M40 and M47 were injected bilaterally. Monkey M43 was used as the control for the effects of the viral vector construct (bilateral control monkey). Monkeys M24, M34 and M35 were used as control for doxycycline administration. Animal care and experimental procedures were performed in accordance with the ILAR's Guide for the Care and Use of Laboratory Animal, and the European legislation (Directive 2010/63/EU) and were approved by the Ethical Committee of KU Leuven. Animal housing and handling were in accordance with the

recommendations of the Weatherall report, allowing locomotor behavior, social interactions and foraging. All animals were group-housed (cage size at least 16-32 m³) with cage enrichment (toys, foraging devices) at the primate facility of the KU Leuven Medical School. They were fed daily with standard primate chow supplemented with bread, nuts, raisins, prunes and fruits. The monkeys were exposed to natural light conditions, with 12 hours of additional artificial light throughout the year, independent of the season. The animals received their daily water supply during the experiments until satiated.

Viral vector preparation

To selectively express eTeNT within the VTA to NAc pathway, a highly efficient retrograde gene transfer vector (HiRet) or neuron-specific retrograde gene transfer vector (NeuRet) carrying TRE-EGFP.eTeNT was injected into the nucleus accumbens and AAV-CMV-rtTAV16 was injected into the VTA, following the method of Kinoshita et al. (2012). The injected vectors, including the control vectors, are shown in Supplemental Table 2.

FuG-B2 (HiRet)- and FuG-C (NeuRet)-TRE-EGFP.eTeNT vectors were prepared as previously described in (Kato et al., 2011; Kinoshita et al., 2019; Kinoshita et al., 2012; Kobayashi et al., 2016). Briefly, the packaging plasmids (pRSV-Rev and pMDLg/pRRE), envelope plasmid (pCAGGS-FuG-B2 or C), and transfer plasmid (pLenti-TRE-EGFP.eTeNT-PEST-WPRE) were transfected into HEK293T cells using the calcium phosphate precipitation method. Eighteen hours after transfection, medium was replaced with fresh medium, and cells were incubated for 24 h. Crude viral vector particles were harvested by centrifuging the cultured medium (6000 x g for 16-18h), and purified by a Sepharose Q FF ion-exchange column (GE Healthcare).

Peak fractions containing the vector particles were collected and concentrated by ultrafiltration. The copy number of the viral RNA genome was determined by using a Lenti-X qRT–PCR titration kit (Clontech).

AAV2-CMV-rtTAV16 vectors were packaged as previously described in (Kobayashi et al., 2016). Briefly, the packaging plasmids (pAAV-RC2 and pHelper) and transfer plasmid (pAAV-CMV-rtTAV16) were transfected into HEK293T cells. After incubation, harvested cells were lysed and purified by serial ultracentrifugation with cesium chloride. The purified particles were dialyzed with 0.001% Pluronic-F68 Solution (Sigma-Aldrich) in PBS, and then concentrated by ultrafiltration. The copy number of the viral genome (vg) was determined by the TaqMan Universal Master Mix II (Applied Biosystems).

Viral vector injection procedure

Anesthesia was induced by an intramuscular injection of ketamine (100 mg/ml Nimatek; Eurovet Animal Health B.V.) and medetomidine hydrochloride (1 mg/ml; Orion Pharma). Monkeys were kept under deep anesthesia with isoflurane (1.5%) inhalation or propofol (i.v. 20 mg/ml Diprivan; Novaplus). During surgery, vital functions were constantly monitored (heart rate, respiration rate, blood pressure, oxygen saturation, end-tidal carbon dioxide and body temperature). To target the VTA and NAc, monkeys were first fitted with MR-compatible chambers guided by CT and T1-weighted imaging (see (Arsenault et al., 2014) for details). Before injection, craniotomies within the chambers targeting VTA and NAc were performed and the guide tube lengths and position were determined using T1-weighted MR images. The accuracy of the guide-tube positioning was verified using peri-operative MR scanning.

A plastic grid (Crist Instruments) was then inserted into the cylinder, and a 22-gauge guide tube (fused silica, Plastics One) was used to guide the trajectory of the injection needle. For the injection, 27G (VTA; AAV construct: $1.6 - 3.0 \times 10^{13}$ vg/ml) and 28G (NAc; Hi-Ret/Neu-Ret construct: $5.3 - 39 \times 10^{11}$ copies/ml) Hamilton needles (140 mm; point style 2; no hub) were attached to polytetrafluoroethylene tube. Before injection, a 2-minute rest interval was implemented to stabilize the tissue and needle. Subsequently 0.50-0.75 μ l (0.1 μ l/min rate) was injected using a Hamilton syringe (10 μ L, Model 701, Cemented NDL, 26sG, 2 in, point style 2) attached to a micro-infusion pump (Legato® 130 Syringe Pump, KdScientific). This was followed by another 2 minutes rest interval after each injection and then the needle was slowly lowered to the next injection site. The number of injection sites was dependent on the target region (Supplemental Table 2). Once the injections were completed, the craniotomy was closed with a silicone elastomer (Kwik-Sil™, World Precision Instruments). Postoperatively, the animals were treated with intramuscular analgesics (5mg/kg Meloxicam, Boehringer Ingelheim) and antibiotics (1g Cefazoline, Sandoz) and kept under quarantine for 10 days.

Doxycycline administration

Doxycycline hyclate ($\geq 98\%$ doxycycline hydrochloride hemiethanolate hemihydrate, Sigma-Aldrich, #D9891; dox) was dissolved in an 11.3% sucrose solution (apple juice; Minute Maid) and administered orally before training (15 mg/kg) approximately 6-14 weeks after viral vector injection (Figure 1B). Each cycle of dox administration lasted 17-29 days for the behavioral and resting-state fMRI experiments. Specifically, M34 underwent one round of dox administration (with a total of 16 days dox-off and 21 days dox-on; in short: 16/21); M24 (two rounds of 19/19

and 19/19 days for cycle one and two, respectively); M30 (two rounds of 22/22 and 21/21 days, respectively); M35 (two rounds of 5/19 and 19/19 days); M36 (two rounds of 23/23 and 18/18 days); M43 (two rounds of 18/18 and 9/9 days); M47 (three rounds of 7/19; 21/29; 26/26 days, followed by 26 days of dox-off); and M40 which participated in the resting-state fMRI and two behavioral experiments (seven rounds of 25/25; 22/22; 21/21; 17/17; 18/18 18/18 days, followed by 28 days of dox-off). Before a dox-off cycle, monkeys were first trained until they reached stable performance (either for fixation during the resting-state fMRI experiments, or behavioral performance on the reinforcement learning or motivational task), which lasted 15 days on average. There were also 9 days (average) of rest between the dox-on and dox-off periods within a cycle and between the subsequent cycles. Note that the last cycle of M43 (for histology only) was shorter than the other cycles, but this was still sufficient to double-transduce a reasonable number of neurons. This shorter cycle was decided to finish the histology within a reasonable time frame. The pharmaco-kinetic and dynamic profile after oral administration of doxycycline has previously been described in macaque by (Kinoshita et al., 2012).

Histological assessment

At the end of the experiments, three monkeys (M30, M36 and M43) were deeply anesthetized with ketamine (100mg/kg, intramuscular, Nimatek; Eurovet Animal Health B.V.) and euthanized using an overdose of pentobarbital (Nembutal 60 mg/ml, intravenous, 30mg/kg). The animal was then transcardially perfused using isotonic 0.05M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in a 0.1M phosphate buffer (PB, pH 7.4). Subsequently, the brain was removed from the skull, cryoprotected, and then, sectioned with a sliding microtome (RetorotomeREM-710,

Yamato, Asaka, Japan; 40- μ m-thick coronal (M36 and M43) or parasagittal (M30) sections).

For immunohistochemistry of double-infected neurons, sections were incubated in 1:2000 rabbit polyclonal anti-GFP (Invitrogen, A11122) or 1:5000 polyclonal rabbit anti-RFP (Rockland Immunochemicals, Inc., #600-401-379) in 2% NGS/PBS-T, followed by biotin goat anti-rabbit IgG (1:200; Vector Laboratories BA-1000). After slices were incubated with vectastain elite ABC kit (Vector laboratories) they were visualized with diaminobenzidine (DAB) containing Nickel ammonium sulfate (0.01% DAB, 1.0% Nickel ammonium sulfate, 0.0003% H₂O₂). Then sections were mounted onto gelatin-coated slide glass and counterstained with neutral red.

For double staining with anti-tyrosine hydroxylase (TH) and fluorescent protein, sections were incubated with monoclonal mouse anti-TH (1:500; Sigma-Aldrich T2928) and 1:2000 polyclonal rabbit anti-GFP or 1:5000 polyclonal rabbit anti-RFP. Subsequently, sections were visualized with AF594 goat anti-rabbit IgG (1:200; Invitrogen, A-11012) and AF488 Goat anti-mouse IgG (1:200; Abcam, ab150117) for mCherry or AF488 Goat anti-rabbit IgG (1:200; Invitrogen, A11034) and AF568 Goat anti-mouse IgG (1:200; Invitrogen, A11031) for GFP.

To check the infection of AAV2-CMV-rtTAV16 and FuG-TRE-EGFP.eTeNT, VTA sections were examined by in situ hybridization. Floating sections were treated with a proteinase K (5mg/ml; 37°C; 30min), rinsed and hybridized with an rtTA or EGFP antisense probe at 60°C overnight. The hybridized probe was then detected using an anti-FITC-HRP (1:4000) and alkaline-phosphatase-conjugated anti-digoxigenin antibody (1:1000) followed by nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP; 1:50) colorization. The detailed protocol for dual probe in situ hybridization is freely available online (www.nibb.ac.jp/brish/indexE.html).

Stained slices were observed and photographs were taken using fluorescent microscopes (Akioplan 2, Zeiss, Oberkochen, Germany; BZ-X710; Keyence, NJ, USA) and a confocal microscopy (A1R, Nikon). The number of GFP or mCherry-positive cells was counted in every sixth section (M43; Figure S1). The number of cells was then multiplied by six to estimate the total number of VTA transduced neurons. Three-dimensional models were constructed using 3D software (Neurolucida 360). In addition, tracing of axons in NAc were performed using a light microscope (BX-51, Olympus, Tokyo, Japan).

Resting-state functional magnetic resonance imaging (rsfMRI)

Scanning procedures followed those described by (Vanduffel et al., 2001). rsfMRI scans were acquired using a 3T PrismaFit scanner and a gradient-echo T2*-weighted echo-planar sequence (40 horizontal slices, in-plane 84 x 84 matrix, TR = 2s, TE = 19ms, 1.25mm³ isotropic voxels). Contrast-to-noise ratio was improved by the use of a contrast agent (monocrystalline iron oxide nanoparticle (Feraheme), ~10mg/kg intravenously administered prior scanning). A transmit-only surface coil and an eight-channel external phased array coil system (3.5cm coil diameters), with offline SENSE reconstruction, and an image acceleration factor of 3 were employed. rsfMRI data was collected from monkeys M30 (unilateral), M36, M40 (bilateral) and M43 (bilateral control). rsfMRI data collection was obtained during the last week of the first dox-off and -on periods. The rsfMRI data were preprocessed in preparation for functional connectivity analysis, according to previously described procedures (Mantini et al., 2011; Vincent et al., 2007), and will only be described briefly. The processing steps included (1) regression of white matter and ventricle signals, and their first derivatives; and (2) regression of three-dimensional motion parameters, and their first

derivatives. The seed-based analysis was performed on anatomically-defined bilateral VTA and NAc ROIs. The average time course signal in the ROI was obtained (across voxels within an ROI), and a whole-brain connectivity map was created and converted to z-scores by the Fisher's r-to-z transformation (Zar, 1998), correcting the degrees of freedom for the autocorrelation in the time series (Shumway and Stoffer, 2006). To determine how functional connectivity was affected by dox administration (dox-on vs. dox-off), a two-sample t-test was performed for all voxels in the brain and thresholded at FDR correction ($p < 0.001$).

For ROI-based analysis, the D99-SL atlas space (Saleem and Logothetis, 2012) was co-registered to each subject's anatomy generating 380 anatomical ROIs. Additional ROIs were constructed for the VTA and NAc in both the left and right hemispheres based on anatomical MRI. The average time course signal for each of the 384 ROIs was calculated (across voxels within an ROI) and the correlation between all possible combinations of ROIs (73,536 combinations) was computed and converted to z-scores by the Fisher's r-to-z transformation (Zar, 1998), correcting the degrees of freedom for the autocorrelation in the time series (Shumway and Stoffer, 2006). To determine how functional connectivity was affected by doxycycline administration (dox-on vs. dox-off), a two-sample t-test was performed for each combination of ROIs and thresholded at FDR correction ($p < 0.05$).

The consistency of the significantly modulated functional connectivity across hemispheres and across the two bilaterally inactivated monkeys (M40 and M36) was measured based on the results of the ROI-based analyses. For the across-monkey consistency test, ROI pairs showing significantly modulated functional connectivity in both hemispheres was determined in each monkey separately ($p < 0.05$, FDR corrected), and the strength of functional connectivity changes between all commonly

803 modulated ROI pairs from the two monkeys were extracted and correlated across ROI
 804 pairs of the two monkeys, resulting in r value of 0.83 ($p = 2.04 \times 10^{-46}$). To test whether
 805 the same results can be obtained in individual hemispheres, the same across-monkey
 806 tests (for the bilaterally inactivated subjects) were also conducted for functional
 807 connectivity between ROI pairs of the same hemisphere, thus for the left and right
 808 hemisphere separately. Functional connectivity data of the two monkeys were pooled.
 809 The strength of the functional connectivity changes between all commonly modulated
 810 ROI pairs (of the same hemisphere) were correlated between the two hemispheres,
 811 resulting in an r value of 0.92 ($p = 8.28 \times 10^{-40}$). The same tests were also conducted
 812 between bilaterally (M36 and M40) and unilaterally inactivated (M30) monkeys in each
 813 single hemisphere, to quantify whether the functional connectivity changes were the
 814 same or different across bilaterally and unilaterally inactivated monkeys. When single
 815 hemispheres were compared between M36 and M40, we obtained r values of 0.92 (p
 816 $= 2.75 \times 10^{-15}$) and 0.74 ($p = 1.1 \times 10^{-9}$) for the right and left hemisphere, respectively.
 817 However, when correlating functional connectivity changes across the unilateral (M30)
 818 and bilateral eTeNT animals (M36 and M40), the r values dropped to 0.04 ($p = 0.67$)
 819 for the left hemisphere and -0.10 ($p = 0.37$) for the right hemisphere. These results
 820 suggest that dox induced highly similar common modulations in functional connectivity
 821 across monkeys and hemispheres in the bilaterally inactivated monkeys, but dissimilar
 822 common modulations between bilaterally and unilaterally inactivated monkeys.
 823 To quantify the general effect of inactivation of the VTA-to-NAc connection on resting-
 824 state functional connectivity, we counted all significantly modulated functional
 825 connections and color-coded positive (functional connectivity with dox > without) and
 826 negative modulations differently. To estimate the 95% confidence intervals of the
 827 number of ROI combinations displaying modulations (dox-on vs. dox-off) in functional

connectivity, bootstrapping was implemented. For each subject, fMRI runs during the dox-on and dox-off periods were randomly sampled with replacement. Using this sample, a whole-brain connectivity map was computed as described above and the number of significantly modulated ROI combinations (at FDR correction ($p < 0.05$)) was computed. This was repeated 10,000 times and a bias-corrected and accelerated percentile method was used to calculate the 95% confidence intervals.

Given consistent ROI-based analysis results across hemispheres in bilaterally inactivated monkeys (M36 & M40), we combined the results of the left and right hemispheres of these two monkeys in the consecutive analyses, and we averaged the z-transformed raw functional connectivity strengths across the two hemispheres. To achieve better visualization of the modulated brain areas, we projected the results of functional connectivity changes induced by the inactivation of the VTA-to-NAc pathway (Figure 6) into anatomical space. To this end, we counted the number of connections from any given ROI that showed a significant change in functional connectivity induced by dox administration, and back-projected this information on inflated representations of the cortex (with an additional indication of subcortical regions). ROI pairs showing increased and decreased functional connectivity were counted separately, and color-coded with different colors (per ROI) in anatomical space. Hot colors indicate functional connectivity changes that were positive (i.e. the average functional connectivity with dox > without) and cold colors reflect the opposite (i.e. functional connectivity with dox < without).

To determine whether the functional connectivity changes between ROI pairs, showing significantly modulated functional connectivity (by dox) in bilaterally inactivated animals, covary dynamically with the strength of the VTA-to-NAc pathway inactivation, we performed an additional analysis. Specifically, we correlated the

functional connectivity change between the VTA and NAc (measured across all resting-state fMRI runs acquired in both monkeys during dox-on and dox-off) with the functional connectivity changes of all ROI pairs (showing a significant dox-induced change in functional connectivity), and compared the correlations between dox-on and dox-off conditions using Fisher's Z test. Z-transformed functional connectivity strengths from each ROI pair were first normalized within each monkey before entering the correlation analysis, thus removing the overall difference in functional connectivity strength across the two monkeys. The results were FDR corrected for multiple comparisons at $p < 0.05$. To visualize these dynamic functional connectivity changes in a comprehensive manner, we used a circular plot (Figure 4B). Since not a single pair showed negative correlations with the functional connectivity change observed in the VTA-to-NAc pathway, we showed the raw functional connectivity changes (dox-on vs. dox-off) of all the significantly correlated connections (FDR corrected for multiple comparisons at $p < 0.05$). The results were also visualized in anatomical space using the same counting method as described above.

Behavioral tasks

Motivation-based decision-making task

Monkeys M35, M40 and M47 were first trained for a passive fixation task, followed by a motivation-based decision-making paradigm (Figure 7A). On a given trial, monkeys had to choose between 2 cues, randomly selected from a pool of 5 cues, that differed in the effort required to receive a reward (duration of fixation onto the cue) and the amount of juice delivered upon correct trial completion (Figure 7B). Each of the 5 cues (A-E) was associated with a unique fixation duration and cues requiring longer fixation periods were paired with larger amounts of juice (Figure 7C, Figure S6A). Trials were deemed correct and rewards were delivered if fixation was maintained within the

cue window for the specified time. All cues were equalized for size ($0.7^\circ \times 0.7^\circ$ square) and luminance (11 cd/m^2).

Individual trials of this task began with a 1000 ms period in which the animal had to maintain fixation on a central point ($0.7 \times 0.7^\circ$ bright white square). Thereafter, two randomly selected cues were presented at two distinct, randomized locations. The randomized cue locations were positioned on a 7° radius circle at either four (angle = 0° , 90° , 180° or 270° ; M35 - cycle 1; M40 - all cycles; M47 - cycle 1) or eight (angle = 0° , 45° , 90° , 135° , 180° , 225° , 270° or 315° ; M35 - cycles 2-4; M47 - cycle 2-4) possible locations. After cue presentation, animals selected a cue by making a saccade onto that cue. To receive a juice reward, monkeys had to maintain fixation at that location for a period of time dependent on the selected target cue. When the monkeys moved their gaze outside the 1° central fixation window during the fixation period, the trial was classified as an abort trial. If a cue was selected but fixation was not maintained within the $1.5 \times 1.5^\circ$ window surrounding the cue, the trial was designated as uncompleted. Before data collection began, window bias was assessed. In case of a strong position bias (e.g. 0% selection of left upper quadrant) biased positions were removed from the task before the start of the experiment.

Reinforcement learning task

Monkeys M24, M30, M34, M36, M40 and M43 performed a probabilistic reinforcement learning task (Figure 8A). Each trial started with a short fixation period (1000 - 1500 ms) followed by the simultaneous presentation of 2 visual cues at different locations (geometric shapes filled with solid colors used in (Arsenault et al., 2014)). Each cue was positioned in either the left or right visual field at 9.5° eccentricity along the horizontal meridian. After the cues were presented, the animals selected a visual

cue by making a saccade onto one of the cues. Juice rewards were then delivered based on the reward probability of the selected cue. Each cue was associated with a distinct reward probability (either 2/3 or 1/3 of trials rewarded). After a randomized number of trials (200, 300 or 400 trials), the reward probabilities associated with the cues were switched. Each run consisted of 900 trials and contained 2 reward-probability switches with one pair of cues (Figure 8B). During each run, the reward probability, color and shape were randomly assigned to a pair of cues (cue A and cue B). Within each cycle, cue position was also randomized across trials.

Sigmoid fitting: reinforcement learning task

Preference index data from a window surrounding the cue-reward probability switch (200 trials before, 200 trials after) was fit to the following sigmoid function using

nonlinear regression: $Preference\ Index = \frac{min + (max - min)}{1 + 10^{(x - x_{50}) * m}}$ where x = trial number, min = minimum preference index, max = maximum preference index, x₅₀ = trial number in which preference index equals midpoint between min and max, m = slope of sigmoid.

To estimate 95% confidence intervals of the min, max, x₅₀, m parameters of the sigmoid function bootstrapping was performed. Runs from the dox-off and the dox-on periods were randomly sampled with replacement and the parameters of the sigmoid function were then estimated. This was repeated 10,000 times and used to determine the 2.5% and 97.5% quantile values from the resulting parameter distribution to indicate the confidence interval.

Indirect actor reinforcement learning model: reinforcement learning task

Trial-by-trial cue selection behavior data acquired during the reinforcement

learning task was modeled using an indirect actor model (Evers et al., 2017). Separate models were constructed for the bilateral (M36, M40) and the control animals (M24, M34) and for the dox-on and the dox-off periods. In the indirect actor model, the mean expected pay-off (i.e. action value) for each cue i ($\hat{m}_{i,t}$) where $i = \{A, B\}$ and the variance of these pay-offs ($\hat{\sigma}_{i,t}^2$) was determined. Here the expected pay-off distribution ($\hat{m}_{i,t}^{post}, \hat{\sigma}_{i,t}^{2 post}$) for cue i on trial t was described as: $\hat{m}_{i,t}^{post} = \hat{m}_{i,t}^{pre} + \varepsilon_t \delta_t$ and variance $\hat{\sigma}_{i,t}^{2 post} = (1 - \varepsilon_t) \hat{\sigma}_{i,t}^{2 pre}$ with prediction error $\delta_t = r_t - \hat{m}_{i,t}^{pre}$ and learning rate $\varepsilon_t = \frac{\hat{\sigma}_{i,t}^{2 pre}}{\hat{\sigma}_{i,t}^{2 pre} + \hat{\sigma}_0^2}$ and standard deviation of score σ_0 . The choice of action of the subjects was modeled by a softmax rule. The probability for choosing cue i on trial t is given by: $P_{i,t} = \frac{\exp(\beta \cdot \hat{m}_{i,t}^{pre})}{\sum_j \exp(\beta \cdot \hat{m}_{j,t}^{pre})}$ with exploration parameter β .

Each model was fit to cue selection behavior for the last third of the dox-off and dox-on periods (e.g. if dox-off 1 contained 15 days; data from the last 5 days were included) for the bilateral and control groups. For each indirect actor model, all parameters were taken freely to maximize the log-likelihood of the observed choices. Higher learning rate values indicate that the expected pay-offs are determined by more recent experience with each cue. Larger β -values indicate less noisy selections (i.e. increased probability of selecting the cue of higher value). In addition, the negative log likelihoods (NLL) for the fit of each model to the choice data was computed (smaller NLLs denote that the model more closely fits the behavioral data).

To estimate the 95% confidence intervals of the learning rate, exploration parameter and NLL bootstrapping was employed. For the dox-off and dox-on periods of each group (bilateral and control), runs were randomly sampled with replacement and parameters of the indirect actor model were estimated. This was repeated 10,000 times and used to determine the 2.5% and 97.5% quantile values from the resulting

parameter distribution to indicate the confidence interval.

Statistical analysis

All the behavioral data are reported as sample mean $\pm$ standard error of the mean (SEM) across days. Data included for statistical analysis and indicated as dox-off and dox-on in the figures was exclusively from the last third of each data set. Before statistical comparison of the dox-off and dox-on periods, the data were checked for normal distribution. As the assumption of normal distribution was not met, comparisons between doxycycline groups was performed using a Wilcoxon rank-sum test. Here we test that the median of the dox-off sessions is the same as that during the dox-on sessions (null hypothesis $H_0: \tilde{x}_1 = \tilde{x}_2$), with significance threshold (p-value) less than 0.05. For ROI-based functional connectivity analysis, a two-sample t-test ($H_0: \mu_1 = \mu_2$) was performed for each combination of ROIs and thresholded at FDR correction ($p=0.05$). In addition, bootstrapping was used to estimate the 95% confidence intervals of the number of ROI combinations showing increased or decreased functional connectivity. For seed-based functional connectivity analysis, a two-sample t-test ($H_0: \mu_1 = \mu_2$) was performed for all voxels in the brain and thresholded at FDR correction ($p=0.001$). In the reinforcement learning task, bootstrapping was used to estimate the 95% confidence intervals of the parameters of best fit for a sigmoid function. Bootstrapping was also used to calculate the 95% confidence intervals of the learning rate, exploration parameter, and NLL of an indirect actor model of behavior, during the reinforcement learning task.

976

977 **Movie S1. 3D rendering of double-infected neurons in M43. Related to Figures 1**
 978 **and S1.**

979

980 Movie shows all the slices with double-labeled neurons (GFP and mCherry) in

981 M43. Data are as in Figure S1.

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KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
monoclonal mouse anti-TH	Sigma-Aldrich	T2928
polyclonal rabbit anti-GFP	Invitrogen	A11122
polyclonal rabbit anti-RFP	Rockland Immunochemicals	#600-401-379
Polyclonal rabbit anti-TH	Milipore	AB-152
AF594 Goat anti-rabbit IgG	Invitrogen	A-11012
AF488 Goat anti-mouse IgG	Abcam	ab150117
AF488 Goat anti-rabbit IgG	Invitrogen	A11034
AF568 Goat anti-mouse IgG	Invitrogen	A11031
biotin goat anti-rabbit IgG	Vector Laboratories	BA-1000
Bacterial and Virus Strains		
FuG-B2 (HiRet) -TRE-EGFP.eTeNT-EGFP	Section of Viral Vector Development, National Institute for Physiological Sciences	N/A
FuG-B2 (HiRet) -TRE-EGFP.eTeNT-mCherry	Section of Viral Vector Development, National Institute for Physiological Sciences	N/A
FuG-C (NeuRet)-TRE-EGFP.eTeNT-EGFP	Section of Viral Vector Development, National Institute for Physiological Sciences	N/A
FuG-C (NeuRet)-TRE-EGFP.eTeNT-mCherry	Section of Viral Vector Development, National Institute for Physiological Sciences	N/A

AAV2-CMV-rtTAV16	Section of Viral Vector Development, National Institute for Physiological Sciences	N/A
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Doxycycline hydrochloride hemiethanolate hemihydrate	Sigma-Aldrich	#D9891
Nimatek	Dechra	3120060
medetomidine hydrochloride	Orion Pharma	1070499
Diprivan (propofol)	Aspen Pharma	1482280
Dolethal (pentobarbital)	Vetoquinol	1400522
Diaminobenzidine (DAB)	FUJIFILM Wako Pure Chemical Co.	040-27001
Nickel ammonium sulfate	FUJIFILM Wako Pure Chemical Co.	146-01012
proteinase K	Merck	PCR grade #3115887
alkaline-phosphatase-conjugated anti-digoxigenin antibody	Merck	11093274910
nitro blue tetrazolium (NBT)/ 5-bromo-4-chloro-3-indolyl phosphate (BCIP) stock solution	Merck	11681451001
silicone elastomer	World Precision Instruments	KWIK-SIL
Meloxicam	Boehringer Ingelheim	2266781
Cefazoline	Sandoz	1676691
Critical Commercial Assays		
vectastain elite ABC kit	Vector laboratories	PK6100
Deposited Data		
Behavioral data	This paper	
fMRI data	This paper	
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		

Rhesus monkey (Macaca Mulatta)	Biomedical Primate Research Centre, Rijswijk, Netherlands https://www.bprc.nl/nl	
Oligonucleotides		
rtTA antisense probe	National institute of basic biology	
EGFP antisense probe	National institute of basic biology	
anti-FITC-HRP	National institute of basic biology	
Recombinant DNA		
pCAGGS-FuG-B2 (HiRet)	Addgene	Catalog #; 67513
pCAGGS-FuG-C (NeuRet)	Addgene	Catalog #; 67512
Software and Algorithms		
Neurolucida 360	MBF Bioscience, VT, USA	
Matlab	Mathworks, Natick, MA, USA	
SPM toolbox	Wellcome Trust Centre for Neuroimaging	12.0
Other		
Sliding microtome	Yamato Kohki Ind. Co.	RetorotomeREM-710
fluorescent microscopes	Zeiss	Akioplan 2
fluorescent microscopes	Keyence	BZ-X710
confocal microscopy	Nikon	A1R
light microscope	Olympus	BX-51
3T MRI scanner	Siemens	PrismaFit
10 μ L syringe	Hamilton	Model 701
micro-infusion pump	KdScientific	Legato [®] 130 Syringe Pump

Figure 2 LH

RH

Figure 4

A

B

C

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Figure 5

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B

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D

Figure 6

A Figure 7

B

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D

E

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A Figure 8 **B** **C** **D**

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Supplemental Information

Supplemental Data Items

- Figure S1.** Localization, spread and quantification of FP-positive neurons in the ventral tegmental area of M43.
- Figure S2.** Localization of anti-GFP+, GFP-mRNA+ and tTA-mRNA+ cells in M36 and M43.
- Figure S3.** Seed-based resting-state functional connectivity for NAc and VTA.
- Figure S4.** Matrix of pairwise dox induced changes in functional connectivity.
- Figure S5.** Changes in functional connectivity that correlate with changes in connection strength between VTA and NAc.
- Figure S6.** Motivation-based decision making.
- Figure S7.** Reinforcement learning.

- Table S1.** Subject and experimental characteristics.
- Table S2.** Region of interest (ROI) labels for functional connectivity analysis.
- Table S3.** Statistical analysis (p-values) Motivation-based decision-making task

- Data S1:** Numerical data related to main and supplemental figures

- Movie S1.** 3D rendering of double-infected neurons in M43

Fig. S1

Fig. S2

Fig. S3

bilateral (dox on vs. off)

bilateral (dox on vs. off)

Fig. S4

All pairs

Fig. S5

Fig. S6

Supplemental Information titles and legends

Figure S1. Localization, spread and quantification of FP-positive neurons in the ventral tegmental area of M43. Related to Figure 1. Schematic of anti-FP immunohistochemically stained 40 μm sections. The red boxes indicate the sections stained for mCherry (vector was injected in the right hemisphere (RH)), while the unboxed adjacent sections were stained for green fluorescent protein (GFP; vector was injected in the left hemisphere (LH)). The numbers indicate distances in μm with respect to VTA section 0. The exact number of FP+ cells (labeled as green or red dots) in each section is shown in the histograms bellow (left: GFP; right: mCherry). Note. Size of the dots do not correspond with the actual size of the positive-labeled neurons, but indicate their location. Scale bar, 10 mm.

Figure S2. Localization of anti-GFP+, GFP-mRNA+ and tTA-mRNA+ cells in M36 and M43. Related to Figure 1. **(A and D)** Contour example section with corresponding immunohistochemically GFP+ neurons (GFP+ IHC) in green and antisense GFP-mRNA+ neurons (GFP+ ISH) in yellow for M36 (A) and M43 (D). **(B and E)** Neutral red counterstained anti-GFP positive (labeled in black) section with inset (square; dashed lines) on target (VTA) location for M36 (B) and M43 (E). **(C and F)** Enlargement of insets shown in panel B and E, respectively. The left panel shows GFP+ IHC labeled neurons in VTA. For M43 (right panel (F)), and for M36 (middle and rightmost panels (C)), antisense GFP-mRNA+ and tTA-mRNA+ neurons (dark blue dots) are shown. Examples of stained neurons are indicated by a black arrow. Sections in panel C (middle and right) are adjacent to section displayed in panel A, B and left panel of C. Scale bars are 10 mm (A, D), 2 mm (B, E) and 500 μm (C, F), respectively.

Abbreviations: immunohistochemistry (IHC), ISH (in situ hybridization), LH (left hemisphere), RH (right hemisphere) and VTA (ventral tegmental area).

Figure S3. Seed-based resting-state functional connectivity for NAc and VTA.

Related to Figure 4. Doxycycline induced changes in functional connectivity of NAc **(A)** and VTA **(B)** (M36, n=37 runs/period; M40, n = 40 runs/period, bilateral eTeNT, dox-on vs. dox-off, FDR corrected $p < 0.001$). Abbreviations: 1-2 (somatosensory area 1 and 2), 12 (prefrontal area 12), 13 (prefrontal area 13), AL (anterior lateral, belt of the auditory cortex), CA (cornu ammonis), Cb (cerebellum), CC (corpus callosum (splenium)), Cd (caudate nucleus), cla (claustrum), EC (entorhinal cortex, caudal division), ECL (entorhinal cortex, caudal limiting division), F5 (agranular frontal area F5 (6Va/6Vb)), FST (floor of superior temporal area), GP (globus pallidus), GPe (globus pallidus external), Hy (hypothalamus), lai (intermediate agranular insula area), lai (lateral agranular insula area), ic (internal capsule), iPA (intraparietal sulcus associated area in the STS), LGN (lateral geniculate nucleus), mcp (middle cerebellar peduncle), Md (medulla), ml (medial lemniscus), MT (middle temporal area), NAc (nucleus accumbens), NBM (nucleus basalis of Meynert), PN (pontine nucleus), PrCO (precentral opercular area), ProS (prosubiculum), Pu (putamen), R (rostral, core region of the auditory cortex), RM (rostromedial, belt region of the auditory cortex), RPB (rostral parabelt region of the auditory cortex), RTL (lateral rostrotemporal, belt auditory cortex), RTp (rostrotemporal, polar part), SII (secondary somatosensory area), SN (substantia nigra), STG (superior temporal gyrus), STGr (rostral superior temporal gyrus), STN (subthalamic nucleus), Sub (subiculum), TAa (temporal area TAa (STS dorsal bank area)), TEa (anterior temporal area (STS ventral bank area)), TEM (temporal area TE, medial part (STS ventral bank)), TEO (temporal area TE, occipital

part), TEp (temporal area, posterior), TEpd (dorsal subregion of posterior PE), TF (temporal area of the parahippocampal cortex), TFO (temporal area, occipital part of the parahippocampal cortex), TGa (agranular part of the temporal pole), TGdd (dysgranular part of the dorsal temporal pole), TGgd (granular part of the dorsal temporal pole), V1 (primary visual cortex), V2 (visual area 2), V3 (visual area 3), V3v (visual area 3, ventral part), V4 (visual area 4) and VTA (ventral tegmental area).

Figure S4. Matrix of pairwise dox induced changes in functional connectivity.

Related to Figure 4. Change in functional connectivity (between dox-on vs. dox-off) for all left and right hemisphere ROIs in the bilateral eTeNT subjects (M36 and M40) **(A)**, control monkey (M43) **(B)**, and the unilateral (left hemisphere) eTeNT subject (M30) **(C)**. A two-sample t-test was performed for each combination of ROIs and thresholded at FDR correction $p < 0.05$. The numbers correspond to ROIs listed in Table S2. LH = left hemisphere; RH = right hemisphere.

Figure S5. Changes in functional connectivity that correlate with changes in connection strength between VTA and NAc. Related to Figure 4. Scatter plots of all ROI pairs showing significant dox induced changes in functional connectivity that were correlated with dox induced changes in functional connectivity between VTA and NAc. Each red and blue dot represents normalized functional connectivity of a resting state fMRI run (from bilateral eTeNT animal M36 or M40) acquired during dox-on and dox-off sessions, respectively. Linear regression lines are shown in red and blue for the dox-on and dox-off sessions, respectively. Abbreviations see Table S2.

Figure S6. Motivation-based decision making. Related to Figure 7. **(A)** Relationship between fixation duration and reward for each cue in each monkey and doxycycline

cycle (cycle number indicated in parentheses). **(B)** Mean normalized cue preference for all trials. **(C)** Mean proportion of aborted (i.e. uncompleted) trials for the high effort_(cue D+E) vs. low effort_(cue A+B). **(D)** Mean percentage of total cue fixation time during uncompleted trials. **(E)** The daily working time (i.e. hours/day monkey is willing to perform the task) and reaction time. **(B-E)** Values were computed from last third of dox-off and dox-on periods. Error bar denotes SEM across days (M40, n = 20/14; M47, n = 21/19; M35, n = 7/10 (days dox off/on)). Wilcoxon rank-sum test used to compute significance (“*” denotes p<0.05).

Figure S7. Reinforcement learning. Related to Figure 8. **(A)** Min, max, midpoint and slope of sigmoid model and **(B)** learning rate, and **(C)** exploration parameter estimates from indirect actor models for bilateral eTeNT (M36, n = 18/22; M40, n= 39/39 runs off/on) and control (M24, n = 30/19; M34, n = 13/15 runs off/on) monkeys. **(D)** Daily work time ((i.e. hours/day monkey is willing to perform the task) and **(E)** reaction time for all monkeys (M36, n = 7/9; M40, n = 10/11; M24, n = 8/9; M34, n = 3/4 (days dox off/on)). Data from last third of dox-off and dox-on periods. (a-c) Error bars denote 95% confidence intervals determined by bootstrapping (10,000 iterations). (d-e) Error bars denotes SEM across days. A Wilcoxon rank-sum test was used to compute significance (*p<0.05).

Supplemental Tables

Table S1. Related to Figure 1.
Subject and experimental characteristics

	eTeNT monkeys								control monkeys					
	unilateral		bilateral						bilateral control			doxycycline control		
	M30		M36		M40		M47		M43			M24	M34	M35
gender	♀		♂		♂		♂		♂			♀	♂	♂
age (year)	6		5		4		4		5			7	6	7
weight (kg)	5.0		6.0		6.0		6.7		7.0			4.5	6.5	9.0
vector type	AAV2	FuGC	AAV2	FuGC	AAV2	FuG-B2	AAV2	FuG-B2	AAV2	FuG-B2	FuG-B2	-	-	-
vector FP-tag	-	EGFP	-	EGFP	-	EGFP	-	EGFP	-	EGFP	mCherry	-	-	-
vector titer ^a	1.4e ¹⁰	3.2e ¹²	2.2 ¹⁰	3.9e ¹²	2.5e ¹⁰	5.3e ¹¹	1.6e ¹⁰	2.3e ¹²	2.5e ¹⁰	5.1e ¹¹	5.4e ¹¹	-	-	-
injection site	L VTA	L NAc	L/R VTA	L/R NAc	L/R VTA	L/R NAc	L/R VTA	L/R NAc	L/R VTA	L NAc	R NAc	-	-	-
volume (μl)	10	15.3	9	12.5	9	12.5	13.5	18.75	9	12.5	12.5	-	-	-
rate (μl/ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-
#tracks	5	5	6	5	6	5	6	5	6	5	5	-	-	-
#depths/tracks	4	5	3	5	3	5	3	5	3	5	5	-	-	-
task	RLT		RLT		RLT & MBD		RLT & MBD		-			RLT	RLT	MBD
data	b, f, h		b, f, h		b, f		b		f, h			b	b	b
waiting time ^b	47 days		66 days		28 days		63 days		159 days			-	-	-
† time ^c	146 days		183 days		-		-		268 days			-	-	-

Abbreviations: f (resting-state functional magnetic resonance imaging); h (histology); b (behavior); RLT (reinforcement learning task); MBD (motivation-based decision making task); AAV (adeno-associated viral vector); D (depth); EGFP (enhanced green fluorescent protein); eTeNT (enhanced tetanus neurotoxin); FuG-B2 (fusion glycoprotein type B2); FuGC (fusion glycoprotein type C; L (left); mCherry (monomeric red fluorescent protein); NAc (nucleus accumbens); R (right); TRE (tetracycline responsive element); VTA (ventral tegmental area). **Symbols:** ♀ (female); ♂ (male); ^a AAV titer unit vg/μl – FuG titer unit cp/ml; ^b waiting time (i.e. time in-between injection and first doxycycline off data collection point); ^c † time (i.e. time in-between injection and perfusion).

Table S2, Related to Figure 4-6, Supplemental Figures 3-5.
Region of interest (ROI) labels for functional connectivity analysis

#	ROI name (Abbreviation)	#	ROI name (Abbreviation)	#	ROI (Abbreviation)
1	somatosensory areas 1 and 2 (1 2)	65	CA1 subfield of hippocampus (CA1)	-	periamygdaloid cortex 3 (PAC3)
-	somatosensory areas 3a and 3b (3a b)	-	CA2 subfield of hippocampus (CA2)	130	periamygdaloid cortex o/piriform cortex (PACo Pir)
-	superior parietal lobule area (part of 5) (PE)	-	CA3 subfield of hippocampus (CA3)	-	periamygdaloid cortex, sulcal portion (PACs)
-	parietal area PE, medial bank (part of 5) (Pea)	-	CA4 subfield of hippocampus (CA4)	-	parasubiculum (paraS)
5	parietal area PE, dorsocaudal pole (Pec)	-	central nucleus of amygdala (CE)	-	superior parietal lobule area (cingulate sulcus) (PEci)
-	caudal inferior parietal lobule area 7a (Opt)	70	cerebellum (Cerebellum)	-	area PG associated region of the superior temporal sulcus (PGA)
-	rostral inferior parietal lobule area 7b (PFG)	-	caudal lateral, belt of the auditory cortex (CL)	135	inferior pulvinar (PI)
-	area 7m in the medial parietal cortex (PGm)	-	Clastrum (Clastrum)	-	parainsular area (Pi)
-	area 7op, parietal operculum (7op)	-	caudomedial, belt of the auditory cortex (CM)	-	posterior intraparietal area (PIP)
10	periarculate area (or FEF), dorsal subdivision (8Ad)	-	anterior cortical nucleus (COa)	-	piriform cortex (Pir)
-	periarculate area (or FEF), ventral subdivision (8Av)	75	posterior cortical nucleus (COP)	-	lateral pulvinar (PL)
-	dorso-lateral prefrontal area 8Bd (8Bd)	-	caudal parabelt region of auditory cortex (CPB)	140	medial pulvinar (PM)
-	dorso-medial prefrontal area 8Bm (8Bm)	-	dentate gyrus (DG)	-	precentral opercular area (PrCO)
-	dorso-lateral prefrontal area 8Bs, arcuate sulcus upper limb (8Bs)	-	dorsal prelunate area (DP)	-	presubiculum (preS)
15	dorso-lateral prefrontal area 9d (9d)	-	entorhinal cortex, caudal division (EC)	-	prosubiculum (proS)
-	dorso-medial prefrontal area 9m (9m)	80	entorhinal cortex, caudal limiting division (ECL)	-	rostral, core region of the auditory cortex (R)
-	medial prefrontal area 10mc, caudal part (10mc)	-	entorhinal cortex, intermediate division (EI)	145	retroinsula (Ri)
-	medial prefrontal area 10mr, rostral part (10mr)	-	entorhinal cortex, lateral division (caudal part) (Elc)	-	rostromedial, belt region of the auditory cortex (RM)
-	orbital prefrontal area 10o (10o)	-	entorhinal cortex, lateral division (rostral part) (Elr)	-	rostral parabelt region of the auditory cortex (RPB)
20	orbital prefrontal area 11i (11i)	-	entorhinal cortex, olfactory division (EO)	-	rostrot temporal, core auditory cortex (RT)
-	orbital prefrontal area 11m (11m)	85	entorhinal cortex, rostral division (ER)	-	lateral rostrot temporal, belt auditory cortex (RTL)
-	ventro-lateral prefrontal area 12l (12l)	-	agranular frontal area F1, 4 (F1 4)	150	rostrot temporal, polar part (RTp)
-	orbital prefrontal area 12m (12m)	-	agranular frontal area F2, 6DR/6DC (F2)	-	secondary somatosensory area (SII)
-	orbital prefrontal area 12o (12o)	-	agranular frontal area F3, SMA (F3)	-	substantia nigra pars reticulata or compacta (SNr c)
25	ventro-lateral prefrontal area area 12r (12r)	-	agranular frontal area F4, 4C/6Va/6Vb (F4)	-	rostral superior temporal gyrus (STGr)
-	orbital prefrontal area 13a (13a)	90	agranular frontal area F5, 6Va/6Vb (F5)	-	subthalamic nucleus (STN)
-	orbital prefrontal area 13b (13b)	-	agranular frontal area F6 (preSMA)	155	Striatum (Striatum)
-	orbital prefrontal area 13l (13l)	-	agranular frontal area F7, 6DR (F7)	-	subiculum (Sub)
-	orbital prefrontal area 13m (13m)	-	floor of superior temporal area (FST)	-	temporal area TAa, STS dorsal bank (TAa)
30	orbito-medial prefrontal area 14c (14c)	-	gustatory cortex (G)	-	temporal area TEa, STS ventral bank (TEa)
-	orbito-medial prefrontal area 14r (14r)	95	globus pallidus, external/internal segment (GPe/i)	-	dorsal subregion of anterior TE (TEad)
-	area 23a in posterior cingulate cortex (23a)	-	agranular insula (Ia)	160	ventral subregion of anterior TE (TEav)
-	area 23b in posterior cingulate cortex (23b)	-	intermediate agranular insula area (Iai)	-	temporal area TE, medial part TEm, STS ventral bank (TEm)
-	area 23c in posterior cingulate cortex (23c)	-	lateral agranular insula area (Ial)	-	temporal area TE, occipital part (TEO)
35	area 24a in anterior cingulate cortex (24a)	-	medial agranular insula area (Iam)	-	dorsal subregion of posterior TE (TEpd)
-	area 24b in anterior cingulate cortex (24b)	100	posterolateral agranular insula area (Iapl)	-	ventral subregion of posterior TE (TEpv)
-	area 24c in anterior cingulate cortex (24c)	-	posteromedial agranular insula area (Iapm)	165	temporal area of the parahippocampal cortex (TF)
-	medial prefrontal area 25, subgenual cortex (25)	-	dysgranular insula (Id)	-	temporal area, occipital part of the parahippocampal cortex (TFO)
-	area 29 in retrosplenial cortex (29)	-	granular insula (Ig)	-	agranular part of the temporal pole (TGa)
40	area 30 in retrosplenial cortex (30)	-	intraparietal sulcus associated area in the STS (IPa)	-	dysgranular part of the dorsal temporal pole (TGdd)
-	area 31 in posterior cingulate gyrus (31)	105	lateral dorsal nucleus of amygdala (Ld)	-	granular part of the dorsal temporal pole (TGdg)
-	medial prefrontal area 32 (32)	-	lateral geniculate nucleus, mc (LGNm)	170	STS part of the temporal pole (TGsts)
-	area 35 of the perirhinal cortex (35)	-	lateral geniculate nucleus, pc (LGNp)	-	ventral dysgranular part of the temporal pole (TGvd)
-	area 36 of the perirhinal cortex, caudal subregion (36c)	-	lateral intraparietal area dorsal subdivision (LIPd)	-	ventral granular part of the temporal pole (TGvg)
45	area 36 of the perirhinal cortex, temporal-polar subregion (36p)	-	lateral intraparietal area ventral subdivision (LIPv)	-	temporal area of the parahippocampal cortex (TH)
-	area 36 of the perirhinal cortex, rostral subregion (36r)	110	lateral occipital parietal area (LOP)	-	temporal parietooccipital associated area, STS dorsal bank area (TPO)
-	ventro-lateral prefrontal area in the arcuate sulcus, lower limb (44)	-	lateral ventral nucleus of amygdala (Lv)	175	temporo-parietal area (Tpt)
-	ventro-lateral prefrontal area (45a)	-	medial dorsal nucleus dc division (Mddc)	-	ventral tenia tectum (TTv)
-	ventro-lateral prefrontal area in the arcuate sulcus, lower limb (45b)	-	mediodorsal nucleus, pc division (MDpc)	-	visual area 1, primary visual cortex (V1)
50	dorso-lateral prefrontal area in the dorsal bank and lip of the principal sulcus (46d)	-	medial nucleus of amygdala (ME)	-	visual area 2/v23b in posterior cingulate cortex (V2/V23b)
-	prefrontal area in the fundus of principal sulcus (46f)	115	medial geniculate comple, anterodorsal (MGad)	-	visual area 2 (V2)
-	ventro-lateral prefrontal area in the ventral bank and lip of the principal sulcus (46v)	-	medial geniculate complex, mc part (MGmc)	180	visual area V3A (V3A)
-	primary auditory area (A1)	-	medial geniculate complex, posterodorsal (MGpd)	-	visual area 3, dorsal part (V3d)
-	anterior amygdaloid area (AAA)	-	medial geniculate complex, ventral (MGv)	-	visual area 3, ventral part (V3v)
55	accessory basal nucleus of amygdala, mc (ABmc)	-	medial geniculate complex, marginal zone (MGz)	-	visual area 4, dorsal part (V4)
-	accessory basal nucleus of amygdala, pc (ABpc)	120	medial intraparietal area (MIP)	-	V4 transitional area (V4t)
-	anterior hypothalamic areas (AHA)	-	middle lateral, belt region of the auditory cortex (ML)	185	visual area 4, ventral part (V4v)
-	anterior intraparietal area (AIP)	-	mammillary nucleus (MN)	-	visual area 6 or PO (V6)
-	anterior lateral, belt of the auditory cortex (AL)	-	medial superior temporal area (MST)	-	visual area 6A, dorsal division (V6Ad)
60	anterior olfactory nucleus, dorsal/medial division (AONd m)	-	middle temporal area (MT)	-	visual area 6A, ventral division or PO (V6Av)
-	anterior olfactory nucleus, lateral division (AONI)	125	nucleus accumbens (NAc)	-	area v23a in posterior cingulate cortex (v23a)
-	basal nucleus of amygdala, intermediate subdivision (Bi)	-	nucleus of the lateral olfactory tract (NLOT)	190	area v23b in posterior cingulate cortex (v23b)
-	basal nucleus of amygdala, mc subdivision (Bmc)	-	olfactory tubercle (OT)	-	ventral intraparietal area (VIP)
-	basal nucleus of amygdala, pc subdivision (Bpc)	-	periamygdaloid cortex 2 (PAC2)	-	ventral tegmental area (VTA)

Abbreviations: CA (cornu ammonis); DC (dorsocaudal); dc (densocellular); DR (dorsorostral); FEF (frontal eye field); mc (magnocellular); pc (parvocellular); SMA (supplementary motor area); STS (superior temporal sulcus).

Table S3, Related to Figure 7E-G and Figure S6B-E.

Statistical analysis (p-values) Motivation-based decision-making task

		bilateral Blockage			control
		M40	M47	Grouped	M35
normalized preference index ^a (correct trials ^b)	cue A	0.0025	0.0172	0.0194	0.4747
	cue B	0.0565	0.0014	1.1317e⁻⁴	0.4173
	cue C	0.4311	0.2668	0.6401	0.9623
	cue D	0.1464	1.992e⁻⁷	1.6213e⁻⁶	0.5362
	cue E	0.0025	0.0062	2.0872e⁻⁴	0.6009
normalized preference index ^a (all trials ^c)	cue A	0.0091	0.0213	0.0113	0.4173
	cue B	0.3186	0.0053	0.0027	0.3148
	cue C	0.3912	0.4165	0.8704	0.6691
	cue D	0.3912	9.4770e⁻⁷	2.4929e⁻⁶	0.5362
	cue E	0.0165	0.0137	6.9161e⁻⁴	0.5362
highest reward cue selections ^d (%)		0.0240	0.0101	0.0146	0.4173
uncompleted trials ^e (%)		0.0112	2.1170e⁻⁵	0.0015	0.6009
% uncompleted trials cue D_E/ cue A_B ^f		0.1149	0.0015	0.0050	0.5362
% fixation for uncompleted trials ^g	cue A	0.0662	3.4153e⁻⁵	0.0124	1.0000
	cue B	0.0087	0.0791	0.0037	0.6009
	cue C	0.0662	0.9784	0.2468	0.8125
	cue D	0.3359	0.6846	0.3725	0.1331
	cue E	0.4109	0.7045	0.9480	0.3638
daily working time ^h		0.3912	0.6067	0.7941	0.7931
reaction time ⁱ		0.2411	0.4813	0.5717	0.4747

^a total cue n selection trials / total selection trials; ^b trials where cue selection time was successfully completed; ^c trials where cue selection time was aborted; ^d total highest cue selection trials / total cue selections; ^e number of times the selection trial was aborted / total selection trials; ^f Mean proportion of uncompleted trials (highest-effort cues vs. low-effort cues); ^g mean percentage of total cue fixation time during the uncompleted trials; ^h total hours/day monkey was willing to perform the task; ⁱ amount of time it took to select a cue. The p-value was obtained using a Wilcoxon ranksum non-parametric test (5% level of significance (i.e., $\alpha=0.05$). All significant results (p-value < 0.05) are marked in bold.

Data S1: Numerical data related to main and supplemental figures

Data related to Figure 7, Motivation-based decision making

			bilateral blockage				control	
Panel C								
			M40		M47		M35	
reward (μl)	cue A		30					
	cue B		76					
	cue C		138					
	cue D		216					
	cue E		280					
fixation duration (s)	cue A		1500					
	cue B		3500					
	cue C		5500					
	cue D		7500					
	cue E		9500					
Panel D								
			M40		M47		M35	
			dox off	dox on	dox off	dox on	dox off	dox on
preference index	cue A	day 1	0.17	0.13	0.09	0.06	0.06	0.06
		day 5	0.24	0.10	0.05	0.05	0.05	0.07
		day 10	0.14	0.15	0.06	0.04	0.07	0.08
		day 15	0.15	-	0.06	0.13	0.11	0.09
		day 20	0.12	-	0.06	-	-	-
	cue B	day 1	0.16	0.11	0.12	0.08	0.16	0.14
		day 5	0.16	0.09	0.09	0.07	0.12	0.14
		day 10	0.12	0.11	0.12	0.07	0.18	0.17
		day 15	0.11	-	0.10	0.16	0.29	0.19
		day 20	0.08	-	0.08	-	-	-
	cue C	day 1	0.24	0.28	0.29	0.25	0.27	0.31
		day 5	0.22	0.28	0.30	0.25	0.29	0.27
		day 10	0.21	0.26	0.26	0.34	0.27	0.31
		day 15	0.28	-	0.30	0.31	0.29	0.27
		day 20	0.28	-	0.29	-	-	-
	cue D	day 1	0.25	0.26	0.28	0.28	0.28	0.28
		day 5	0.19	0.28	0.29	0.29	0.31	0.30
		day 10	0.30	0.27	0.29	0.29	0.27	0.26
		day 15	0.25	-	0.28	0.19	0.26	0.27
		day 20	0.28	-	0.27	-	-	-
	cue E	day 1	0.18	0.22	0.23	0.33	0.23	0.22
		day 5	0.19	0.25	0.28	0.34	0.23	0.22
		day 10	0.23	0.21	0.27	0.27	0.20	0.17
		day 15	0.22	-	0.27	0.21	0.16	0.17
		day 20	0.25	-	0.30	-	-	-
Panel E								
			M40 (mean ± sem)		M47 (mean ± sem)		M35 (mean ± sem)	
normalized preference index	dox off	cue A	1.00 ± 0.05		1.00 ± 0.03		1.00 ± 0.11	
		cue B	1.00 ± 0.06		1.00 ± 0.05		1.00 ± 0.05	
		cue C	1.00 ± 0.02		1.00 ± 0.03		1.00 ± 0.04	
		cue D	1.00 ± 0.03		1.00 ± 0.01		1.00 ± 0.04	
		cue E	1.00 ± 0.04		1.00 ± 0.02		1.00 ± 0.06	
	dox on	cue A	1.27 ± 0.04		1.59 ± 0.10		0.93 ± 0.08	
		cue B	1.67 ± 0.04		1.55 ± 0.08		0.89 ± 0.07	
		cue C	0.98 ± 0.04		1.13 ± 0.06		1.01 ± 0.03	
		cue D	0.93 ± 0.03		0.80 ± 0.03		1.04 ± 0.02	
		cue E	0.84 ± 0.03		0.81 ± 0.07		1.07 ± 0.07	
Panel F								
			M40		M47		M35	
highest reward cue selections (%)	dox off		59.01 ± 1.35		77.62 ± 0.73		64.09 ± 1.56	
	dox on		53.67 ± 0.82		66.79 ± 2.60		66.12 ± 1.39	
Panel G								
			M40		M47		M35	
Uncompleted trials (%)	dox off		23.71 ± 2.23		9.57 ± 0.93		20.66 ± 1.32	
	dox on		30.56 ± 1.76		17.20 ± 1.17		21.81 ± 0.75	

Data related to Figure 8, Reinforcement learning

		bilateral blockage	control
Panel D			
		M36 & M40 (95% CI)	M24 & M34 (95% CI)
learning rate	dox off	0.12 (0.09, 0.14)	0.09 (0.03, 0.16)
	dox on	0.11 (0.08, 0.13)	0.10 (0.05, 0.18)
exploration	dox off	7.57 (6.17, 8.85)	6.52 (3.44, 9.64)
	dox on	7.05 (5.23, 9.01)	6.48 (4.51, 8.97)

Data related to Figure S1, Localization, spread and quantification of FP positive neurons in the ventral tegmental area of M43

		FP+ cell count	
		GFP	mCherry
distance (μm)	0	1	
	40		0
	240	2	
	280		1
	480	3	
	560		4
	720	3	
	760		5
	960	3	
	1000		5
	1200	1	
	1240		10
	1440	4	
	1480		9
	1680	6	
	1720		8
	1920	6	
	1960		8
	2160	6	
	2200		15
	2400	12	
	2440		17
	2640	7	
	2680		36
	2880	7	
	2920		26
	3120	8	
	3160		28
	3360	6	
	3400		26
	3600	9	
	3640		24
	3840	15	
	3880		16
	4080	6	
	2120		12
	4320	7	
	4360		3
	4560	9	
	4600		10
	4800	15	
	4840		5
total number of soma		136	268
estimated number of soma (i.e. Total # x 6)		816	1608

Data related to Figure S6, Motivation-based decision-making

			bilateral blockage			control
Panel A						
			M40	M47		M35
			▲ cycle 1-4	■ cycle 1	● cycle 2-3-4	■ cycle 1-2
reward (μl)	cue A		30	45	33	45
	cue B		76	114	83	114
	cue C		138	207	150	207
	cue D		216	324	234	324
	cue E		280	420	304	420
fixation duration (s)	cue A		1500	1500	900	1500
	cue B		3500	3500	2000	3500
	cue C		5500	5500	3200	5500
	cue D		7500	7500	4300	7500
	cue E		9500	9500	5500	9500
Panel B						
			▲ M40 mean ± sem	■ M47 mean ± sem	● M35 mean ± sem	
normalized PI (correct + uncompleted trials)	dox off	cue A	1.00 ± 0.04	1.00 ± 0.02	1.00 ± 0.11	
		cue B	1.00 ± 0.05	1.00 ± 0.04	1.00 ± 0.05	
		cue C	1.00 ± 0.02	1.00 ± 0.03	1.00 ± 0.04	
		cue D	1.00 ± 0.03	1.00 ± 0.01	1.00 ± 0.03	
		cue E	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.04	
	dox on	cue A	1.17 ± 0.04	1.40 ± 0.08	0.86 ± 0.06	
		cue B	1.08 ± 0.05	1.46 ± 0.08	0.89 ± 0.09	
		cue C	1.00 ± 0.03	1.09 ± 0.05	1.02 ± 0.03	
		cue D	0.97 ± 0.02	0.82 ± 0.02	1.04 ± 0.02	
		cue E	0.92 ± 0.02	0.88 ± 0.05	1.06 ± 0.06	
Panel C						
			▲ M40 mean ± sem	■ M47 mean ± sem	● M35 mean ± sem	
% uncompleted trials cue D_E/ cue A_B	dox off		1.75 ± 0.10	3.20 ± 0.44	4.00 ± 0.34	
	dox on		1.98 ± 0.07	5.01 ± 0.54	3.77 ± 0.24	
Panel D						
			▲ M40 mean ± sem	■ M47 mean ± sem	● M35 mean ± sem	
% fixation time for uncompleted trials	dox off	cue A	39.81 ± 2.31	26.46 ± 0.81	32.56 ± 3.86	
		cue B	30.52 ± 1.91	27.48 ± 3.77	44.48 ± 2.76	
		cue C	33.74 ± 1.54	39.35 ± 1.30	43.24 ± 1.97	
		cue D	32.06 ± 0.86	32.97 ± 1.66	38.36 ± 0.99	
		cue E	30.95 ± 1.21	36.36 ± 1.31	36.73 ± 0.87	
	dox on	cue A	40.38 ± 3.22	35.51 ± 2.16	33.36 ± 3.46	
		cue B	38.80 ± 1.99	33.36 ± 3.33	44.89 ± 2.32	
		cue C	37.62 ± 1.34	40.66 ± 1.92	42.84 ± 2.51	
		cue D	33.23 ± 1.20	34.59 ± 2.36	42.78 ± 2.28	
		cue E	32.54 ± 0.92	35.80 ± 2.34	39.52 ± 2.63	
Panel E						
			▲ M40 mean ± sem	■ M47 mean ± sem	● M35 mean ± sem	
daily working time (hrs)	dox off		1.41 ± 0.12	1.86 ± 0.14	2.89 ± 0.26	
	dox on		1.56 ± 0.11	1.76 ± 0.11	2.91 ± 0.13	
reaction time (s)	dox off		1.01 ± 0.01	1.17 ± 0.01	1.11 ± 0.02	
	dox on		1.03 ± 0.01	1.19 ± 0.02	1.10 ± 0.01	

Data related to Figure S7, Reinforcement learning

			bilateral blockage		control	
Panel A						
			M36 (95% CI)	M40 (95% CI)	M24 (95% CI)	M34 (95% CI)
Sigmoid model value	min	dox off	-0.76 (-0.81, -0.71)	-0.77 (-0.82, -0.71)	-0.88 (-0.92, -0.83)	-0.48 (-0.59, -0.35)
		dox on	-0.71 (-0.76, -0.65)	-0.84 (-0.87, -0.80)	-0.84 (-0.91, -0.74)	-0.59 (-0.67, -0.51)
	max	dox off	0.81 (0.77, 0.85)	0.77 (0.66, 0.76)	0.88 (0.84, 0.92)	0.40 (0.23, 0.55)
		dox on	0.71 (0.66, 0.76)	0.84 (0.80, 0.87)	0.87 (0.80, 0.93)	0.61 (0.50, 0.72)
	midpoint	dox off	217.82 (215.01, 220.83)	216.84 (214.61, 219.43)	221.01 (218.18, 224.23)	53.46 (33.67, 85.68)
		dox on	221.27 (218.04, 224.51)	213.47 (211.89, 215.31)	220.64 (218.05, 223.37)	40.94 (33.29, 48.19)
	slope	dox off	0.10 (0.07, 0.14)	0.10 (0.7, 0.14)	0.08 (0.05, 0.10)	0.04 (0.01, 0.07)
		dox on	0.08 (0.06, 0.10)	0.12 (0.08, 0.17)	0.09 (0.07, 0.12)	0.04 (0.03, 0.06)
Panel B						
			M36 (95% CI)	M40 (95% CI)	M24 (95% CI)	M34 (95% CI)
learning rate	dox off		0.11 (0.09, 0.12)	0.13 (0.12, 0.14)	0.14 (0.12, 0.16)	0.04 (0.03, 0.05)
	dox on		0.09 (0.07, 0.10)	0.12 (0.11, 0.13)	0.15 (0.11, 0.18)	0.06 (0.05, 0.07)
Panel C						
			M36 (95% CI)	M40 (95% CI)	M24 (95% CI)	M34 (95% CI)
exploration	dox off		6.88 (6.04, 7.70)	8.24 (7.64, 8.94)	8.76 (9.80, 7.84)	4.24 (3.28, 5.34)
	dox on		5.80 (5.16, 7.70)	8.32 (7.54, 9.16)	7.92 (6.60, 9.56)	5.02 (4.42, 5.56)
Panel D						
			M36 mean ± sem	M40 mean ± sem	M24 mean ± sem	M34 mean ± sem
daily work time (hrs)	dox off		2.07 ± 0.13	2.74 ± 0.17	2.30 ± 0.19	4.23 ± 0.37
	dox on		2.01 ± 0.10	3.29 ± 0.28	2.16 ± 0.19	3.65 ± 0.13
Panel E						
			M36 mean ± sem	M40 mean ± sem	M24 mean ± sem	M34 mean ± sem
reaction time (s)	dox off		149.84 ± 1.57	162.51 ± 1.55	174.67 ± 3.78	174.29 ± 3.66
	dox on		146.08 ± 2.22	163.89 ± 2.28	182.81 ± 3.59	161.72 ± 1.45

Figure 4, 5 and S5. The data and code are available online (https://gitlab.com/u0100771/vancraeyenest_arsenault_supplementary).

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Supplemental Videos and Spreadsheets
Movie S1.mp4