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Integrating PET with behavioral neuroscience using RatCAP tomography

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Abstract

Behavioral studies are an important part of neuroscience. They allow inferences about the functions of the brain and any internal states and processes it controls. Positron emission tomography (PET) is an *in vivo* imaging technique that provides insights into the mechanisms of neuronal communication. In this review, we focus on some of the contributions of PET to the field of behavioral neuroscience. Small animals typically require anesthesia to remain still during PET imaging, which places a burden on behavioral studies. Our approach integrates PET with behavioral observations using a miniature PET scanner that rats wear on the head, a mobility system to facilitate animal movement and ways to integrate the PET data with behavioral measures. We summarize our studies that assessed spontaneous, self-initiated behavioral activity and dopamine D2 receptor functions simultaneously.

Keywords: awake behaving rat; conscious animal PET; constant infusion; dopamine D2 receptor; positron emission tomography; striatum.

Introduction

Behavioral neuroscientists study the neurobiological substrates of behavior, the mind, and mental disorders. Behavior is usually some form of body movement or the absence of it, such as walking and sleeping. It is under the control of stimuli in the environment but is also governed by mental processes that we cannot directly observe but must infer from behavior, such as mood and motivation. Mental disorders are complex disorders of the mind and behavior.

The time courses of behavior are often very short. For example, to carry out a single arm movement will take less than a second (Schultz, 2007). Complex behaviors, such as those indicating exploration, consist of movement episodes that can continue for several minutes. The frequency at which such episodes occur and their duration will change over time, for example, with increasing familiarity of the environment and decreasing curiosity (Berlyne, 1955, 1966). Thus, the behavior and any underlying mental processes are dynamic, and information may get lost if we do not measure them simultaneously with the corresponding neurobiological processes.

Few techniques allow the simultaneous assessment of behavioral and brain processes. Electrophysiological techniques can monitor neuronal impulse activity during a behavioral task (Schultz, 2007). Optical imaging techniques (also see Du and Pan, this issue) can assess calcium transients in mobile mice (Dombeck et al., 2007). Neurochemical techniques, such as fast-scan cyclic voltammetry and microdialysis, measure local changes in neurotransmitter release (Westerink, 1995; Clark et al., 2010). Positron emission tomography (PET) is a functional neuroimaging technique that non-invasively images the whole brain and is used to assess glucose metabolism, receptor occupancy, and availability of transporters and enzymes in humans and animals (Fowler and Wolf, 1986; Dewey et al., 1993; Kornblum et al., 2000; Laruelle, 2000; Harada et al., 2004; Fowler et al., 2005; Logan et al., 2007; Itoh et al., 2009). Rodents require anesthesia, however, or restraint and behavioral training to remain still in the scanner. Studies under anesthesia are sometimes advantageous, but anesthetics abolish all behavior and alter the neurochemistry as well (De Souza Silva et al., 2007).

Several techniques were developed to minimize the influence of anesthetics on the PET data. The group of S.R. Cherry used ^{18}F-fluorodeoxyglucose (FDG) to study brain metabolic activity in conscious mice (Kornblum et al., 2000). ^{18}F-FDG is a glucose analogue that is taken up by active brain cells and phosphorylated and thus locked inside the cells until radioactive decay (Gallagher et al., 1978). Anesthetics appear to have negligible effects on ^{18}F-FDG data if sufficient time is allowed for uptake in the conscious state before any sedation (Matsumura et al., 2003). Kornblum et al. (2000) subjected mice to different manipulations during a 45-min uptake period in the conscious state. They found, for example, that stimulation of the vibrissae increased the utilization of glucose in the contralateral neocortex and thalamus. ^{18}F-FDG has since been used in a variety of paradigms in studies of epilepsy (Kornblum et al., 2000; Mirrione et al., 2007), stress (Sung et al., 2009), and depression (Jang et al., 2009). Although informative, the results reflect the sum of all metabolic activity over the entire uptake period and thus are not specific to a particular behavioral event or any changes in behavior over the time of the scanning session.

^{11}C-raclopride is a dopamine D2 receptor (DA D2R) antagonist tracer with fast kinetics and a short half-life. Unlike ^{18}F-FDG, ^{11}C-raclopride is a reversible tracer that binds to and then dissociates from its targets. Patel et al. (2008) explored

an application of ^{11}C-raclopride in which tracer distribution was allowed for 30 min in the conscious state before the rats were anesthetized and scanned. In a follow-up study, the authors found that the binding of ^{11}C-raclopride, which was measured under anesthesia, was predicted by a cocaine-induced place preference, which was measured during tracer uptake (Schiffer et al., 2009). This is an interesting approach, although further work is required to assess the degree to which the binding of ^{11}C-raclopride actually reflects the distribution of the tracer in the conscious state.

Methods have also evolved that image the whole brain at the same time that the animal is awake and active. One approach is to measure the position of the head as the animal moves freely within the confines of a small-animal scanner. Head markers and specialized optical tracking equipment are used to measure and remove the effects of motion from the imaging data. Using a commercial small-animal PET scanner and ^{18}F-FDG, tracking methods were applied to moving phantoms, and a rat whose head was free to move but body was restrained (Kyme et al., 2011). Tracking techniques were also used with a related nuclear medicine technique called single-photon emission computed tomography, which was used to image the brains of awake mice (Weisenberger et al., 2005, and in this issue).

A different approach to enable the correlation of simultaneously acquired PET and behavioral data is to have the animal wear a small scanner on its head as it moves (Schulz et al., 2011). A whole new set of tools and methods was developed, including a miniature high-performance PET scanner (rat conscious animal PET or RatCAP), mechanical methods to attach the scanner and facilitate animal mobility, appropriate radiotracer strategies, and ways to integrate the PET data with behavioral measures.

RatCAP tomograph

The RatCAP scanner is a small complete ring of detectors, weighing 250 g, with an inner diameter of 3.8 cm, outer diameter of 8 cm, and axial extent of 2.5 cm (Figure 1A). It is positioned between the eyes and ears of the rat, allowing a normal forward-facing posture while imaging the whole brain with a field of view of 3.8 cm diameter by 1.8 cm axially (Figure 1B). The spatial resolution is <2 mm full width at half maximum across the field of view, and coincidence sensitivity is 0.76% at the center. For details on the development of the tomograph, see Vaska et al. (2004), Junnarkar et al. (2008), Park et al. (2008), and Pratte et al. (2008).

Attachment brackets

The scanner must be rigidly fastened to the rat to prevent motion artifacts in the PET image. Two plastic sockets are fixed on the skull with tissue adhesive and dental cement, using bregma and lambda as reference points (Figure 2A). An animal bracket is then attached to these sockets. In most of our studies so far, we used a short tube of aluminum that slides into the RatCAP scanner (Figure 2A). This method required momentary anesthesia, so that a new bracket was designed to eliminate this step. The new bracket is small and can be worn permanently, and it has a magnetic tip that latches onto a piece on the scanner in one quick maneuver (Figure 2B).

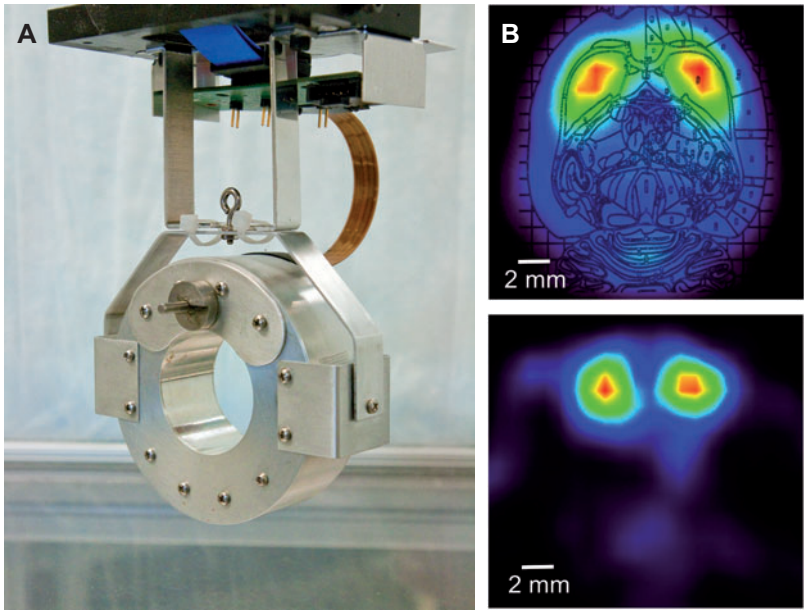

Figure 1 The RatCAP miniature PET scanner.
(A) Photograph of the RatCAP scanner. (B) PET images through the striata of a conscious rat after injection of ^{11}C-raclopride, including a horizontal slice summed over the whole 1-h scan and scaled to a rat brain atlas (top) and a coronal slice from a single dynamic time frame spanning 35–45 min after injection (bottom). Reproduced from Schulz et al. (2011).

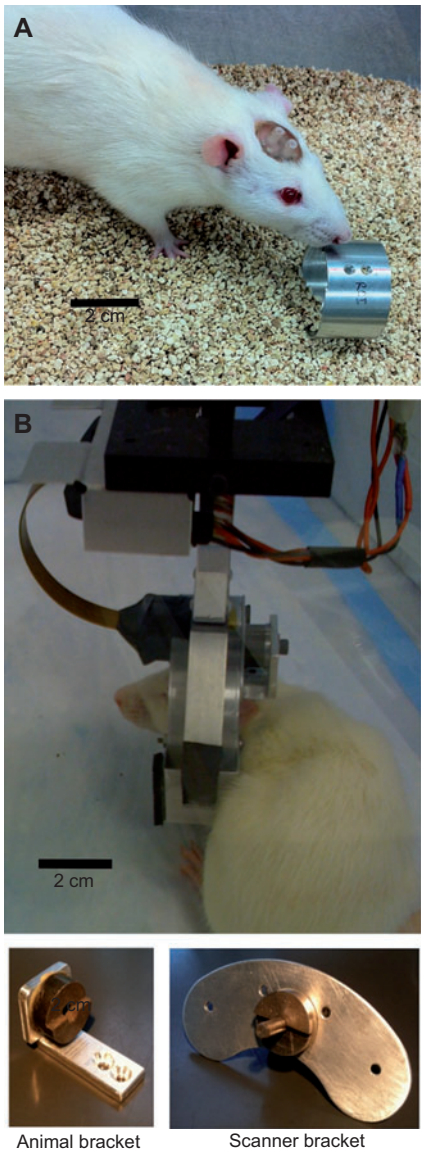

Figure 2 Attachment brackets.
(A) Photograph of a rat showing the plastic sockets on its head after surgery and the tubular sleeve made of aluminum. (B) Photographs showing the improved, self-latching mounting mechanism, including latched brackets (top), detail of animal bracket (bottom left), and detail of scanner bracket (bottom right). Reproduced from Schulz et al. (2011).

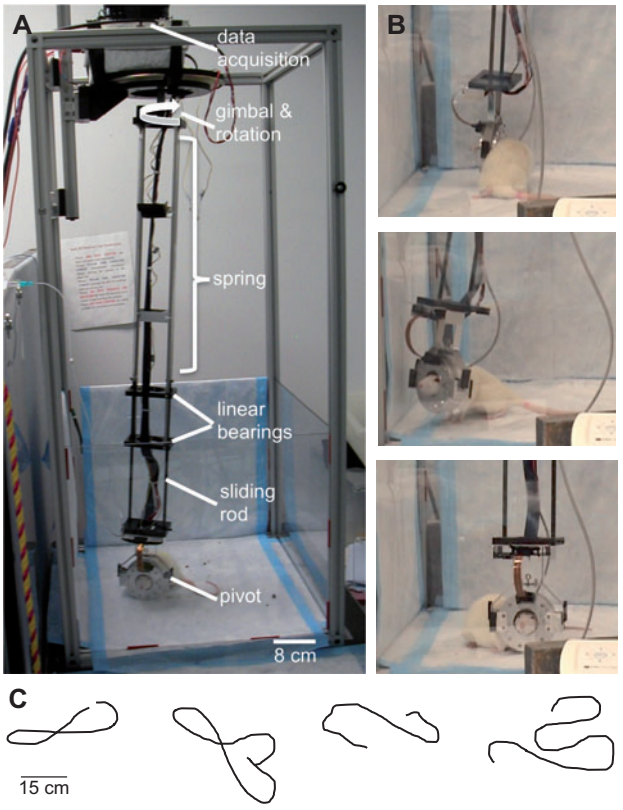

Figure 3 Lightweight telescoping structure allows for animal mobility.
(A) Overview of key components of the animal mobility system. (B) Three video frames exemplifying the typical range of motion of a rat wearing the PET scanner inside the test chamber. (C) Drawings of rat trajectories within the 40×40×40-cm test chamber. The tracks reflect the position of the animal's approximate center. Reproduced from Schulz et al. (2011).

Animal mobility system

As the weight of the scanner is a substantial fraction of the rat's weight, a mechanical system was devised to facilitate animal movement (Figure 3A). The scanner was suspended on long springs that were fastened to a lightweight telescoping structure with linear bearings for vertical motion. This structure was attached at the top to a fixed frame to allow pendulum-like motion across the full chamber. Drawings of rat trajectories in the test chamber are shown in Figure 3C. They illustrate the extent to which the rats can move while wearing the RatCAP. With the current system, movement is restricted mainly by the cables and data acquisition systems. The animals typically move along the floor and can exhibit exploratory rearings, although they are not common.

Our animals appear to adapt well to the system, but we assessed the degree of stress quantitatively by measuring corticosterone in blood as a function of time wearing the scanner.

For blood collection, we used jugular vein catheters, the same implants we also used for tracer infusions during the PET studies. The catheters consisted of Silastic tubing with one end inserted into the vein and the other attached to a back mount cannula connector pedestal that was mounted subcutaneously on the back of the animal. To simulate the time course of our imaging experiments, we collected the blood samples directly from the home cage (baseline), under momentary isoflurane anesthesia, and then while the rat was awake and attached to the RatCAP for 10 min, 1 h, 2 h, and 3 h. The data indicate that although corticosterone levels increased initially, they decreased over time while the tomograph was attached (Figure 4). In one case, they decreased to baseline levels common to animals left undisturbed in the home cage. In the

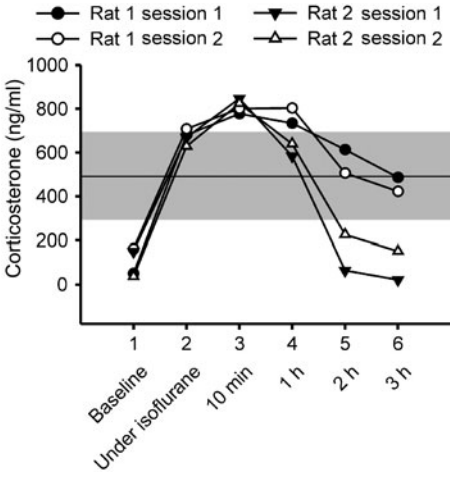

Figure 4 Corticosterone levels.
Corticosterone levels in rat blood plasma (ng/ml) measured at different time points of wearing the RatCAP. The measurements were performed on two occasions (sessions 1 and 2) that were 14 days apart. Sampling took place before and 10 min, 1 h, 2 h, and 3 h after attachment of the RatCAP. The horizontal line (mean) and shaded area (standard deviation) indicate the levels of corticosterone (ng/ml) in a group of animals (n = 10) that underwent transport between animal rooms 30 min before blood sampling from the tail vein. Reproduced from Schulz et al. (2011).

other, the levels were similar to those observed in rats that were transported in their home cages a short distance down the hallway, 30 min before the sample was taken (as indicated by the shaded area in Figure 4). Transport is not typically considered an intervention, although it is accompanied by vigilance and exploration and may also be stressful.

Correlation between PET and behavioral data

We have experimented with two different radiotracer infusion methods. First, we have used the most common method, the bolus injection, in which the tracer dose is given all in less than a minute. With this method, the tracer uptake peaks shortly after the injection, followed by a washout phase. Figure 5A shows the averaged time-activity curves after bolus injections of ^{11}C-raclopride from five awake, behaving rats that were assessed with the RatCAP. As expected, the uptake of ^{11}C-raclopride was higher in the striatum, a brain region rich in D2Rs, than in the cerebellum, which contains few D2Rs and was used as a reference region to account for free and nonspecific or nondisplaceable (ND) uptake (Innis et al., 2007). The striatum-to-cerebellum ratio [BP_{ND}=(STR-CB)/CB=(STR/CB)-1] at equilibrium was taken to indicate specific binding of the radiotracer to D2Rs in the striatum. BP_{ND} was calculated as the average of the ratio over the last five data points on the time-activity curve spanning the last 30 min of the scans and used for correlation with behavioral activity for the same period. Behavioral activity consisted mostly of forward movement but also included orienting head turns and body movement without movement of the head. We found that

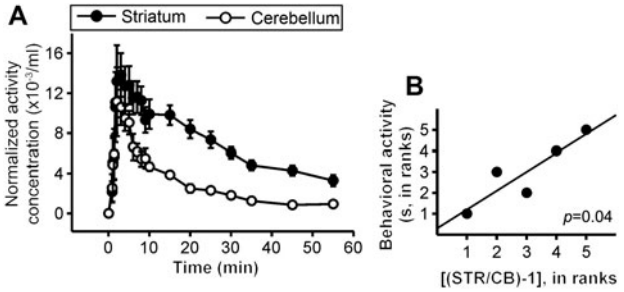

Figure 5 Bolus injections of ^{11}C-raclopride in awake, behaving rats.
(A) Activity concentrations (nCi/ml) of ^{11}C-raclopride over time in the striatum and cerebellum. The data show the average concentrations for five awake, behaving rats. (B) An estimate of specific binding (BP_{ND}=[(STR/CB)-1]) of ^{11}C-raclopride in the striatum was correlated with behavioral activity. All scores indicate ranks, with a rank of 1 indicating the lowest score. STR, striatum; CB, cerebellum. Reproduced from Schulz et al. (2011).

D2R binding by ^{11}C-raclopride was positively correlated with behavioral activity for the animals (Figure 5B). According to the classic competition model, variations in the binding potential (BP) of ^{11}C-raclopride depend on the amount of endogenous ligand that is bound to the receptors (Laruelle, 2000). For example, a higher level of receptor occupancy by endogenous DA translates into a lower BP of ^{11}C-raclopride because fewer binding sites are available for occupancy by the radiotracer. In line with this interpretation, our results indicate that lower levels of D2R binding by DA coincided with higher levels of behavioral activity.

Although we acquired the PET and behavioral data simultaneously with the RatCAP, the time frames for which we computed the PET data were still quite large. In essence, the bolus injection method yields one data point per scan and thus is insensitive to the transient changes in receptor binding that may relate to changes in behavior during the PET scan. By contrast, the bolus and infusion (B-I) method establishes a constant level of radiotracer in tissues and allows the possibility to detect perturbations in this level due to interventions during the scan (Carson, 2000; Garraux et al., 2007; Martin-Soelch et al., 2011). In a human study, a reduction in the BP of ^{11}C-raclopride was found during acquisition of a motor task (Garraux et al., 2007). Interestingly, motor learning was correlated positively and negatively with BP depending on the brain region examined. Using a similar design, Martin-Soelch et al. (2011) compared the effects of a sensorimotor task with a task involving reward on the same PET scan and found that BP_{ND} in the ventral striatum was reduced in the reward condition relative to the sensorimotor condition. The duration of the tasks was approximately 25 min, still a stretch considering the number of behavioral events that can occur during this time period.

We examined the possibility that changes in spontaneous, self-initiated behavioral activity might be reflected in the PET data. Using the B-I method, we first established a steady-state condition of ^{11}C-raclopride (Figure 6A). To graphically display the movement episodes, we summed the durations of

behavioral activity into 2-min bins and cumulated the bins, so that a slope in the behavioral response curve indicated the display of activity and flat regions indicated the absence of activity. We observed that the display of behavioral activity coincided with a slope increase in BP_{ND} and the absence of activity with a slope decrease in BP_{ND} (Figure 6B). We also correlated the differences between the data points on the PET curve with the differences in behavioral activity for the time frames provided by the PET data and again found a positive correlation between the PET and behavioral data (Figure 6C). Transient increases in BP_{ND} (presumably due to a reduction in binding of DA to D2R in the striatum) coincided with increases in behavioral activity during the scan, and transient decreases in BP_{ND} (due to increased binding of DA to D2R) were accompanied by decreases in behavioral activation.

Striatal D2 receptors are found on different neuron types

In the striatum, approximately 95% of neurons are GABAergic medium spiny projection neurons (MSNs; Kita and Kitai, 1988). They give rise to two major striatal output systems: the direct and indirect pathways (Gerfen and Bolam, 2010). The MSN neurons of the direct pathway (approx. half of all MSN neurons) express the DA D1R and the MSN neurons of the indirect pathway express the DA D2R (Gerfen et al., 1990). The modulatory effect of DA released from substantia nigra pars compacta (SNc) neurons on D1 and D2 MSNs is excitatory and inhibitory, respectively, although the net modulatory effect on behavior is an overall increase in movement (Wichmann and DeLong, 2010).

DA autoreceptors (D2auto) are also of the D2 type. They are found in the extrasynaptic space on preterminal regions of striatal SNc neurons (Sesack et al., 1994; Yung et al., 1995). Activation of D2auto inhibits the synthesis and release of DA (Farnebo and Hamberger, 1971; Kehr et al., 1972; Starke et al., 1989) and increases the reuptake of DA *via* DA transporters (DAT) (Schmitz et al., 2003). D2auto activation by low doses of quinpirole inhibits behavioral activity (Eilam and Szechtman, 1989; Depoortere et al., 1996; Usiello et al., 2000; Li et al., 2010). Bello et al. (2011) demonstrated that D2auto are important for normal motor function, food-seeking behavior, and sensitivity to the locomotor and rewarding properties of cocaine. Thus, the positive correlations we obtained with the RatCAP between ^{11}C-raclopride binding and behavioral activity may involve autoreceptor function.

The D2R is also found on cholinergic interneurons in the striatum (Sesack et al., 1994). They account for only 1%–2% of neurons in the striatum (Phelps et al., 1985) but interact with DA neurons in sophisticated ways to influence striatal output (Cragg, 2006). They appear to play an important role

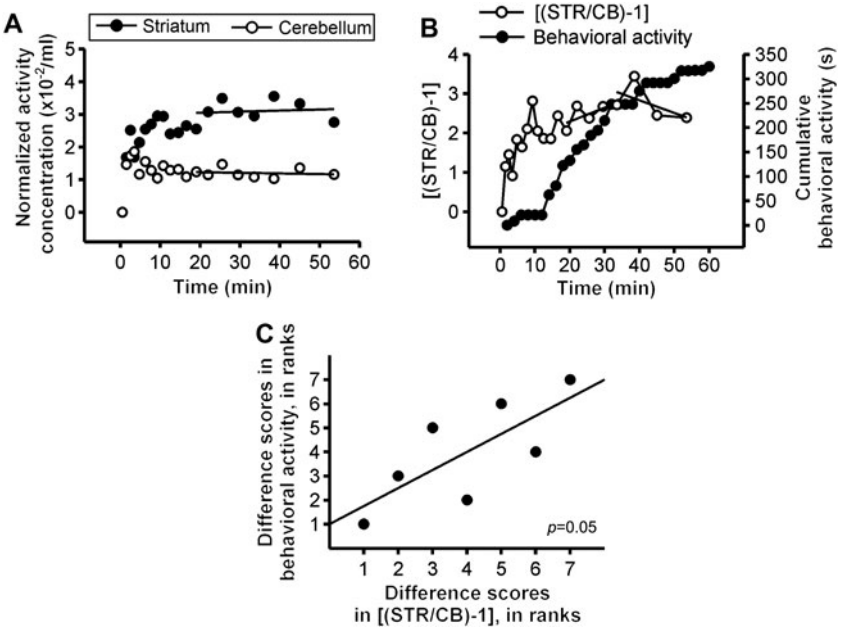

Figure 6 Transient changes in BP_{ND} in relation to behavioral activity during the PET scan.
(A) Activity concentrations of ^{11}C-raclopride (nCi/ml) over time in the striatum and cerebellum of an individual rat using B-I infusion. The relative flatness of the regression line fitting the cerebellum data indicates a steady-state condition of the radiotracer. (B) Depicted are an estimate of specific binding (BP_{ND}=[(STR/CB)-1]) of ^{11}C-raclopride in the striatum and our measure of behavioral activity (cumulated in seconds) from the same animal. STR, striatum; CB, cerebellum. The regression lines show the changes in the slope of the behavioral response curve that reflect the slope changes in [(STR/CB)-1]. (C) Correlation of PET and behavioral data for data points starting at 20 min in B. We subtracted every two successive time points on the PET curve [(STR/CB)-1] and used the difference scores for correlation with the behavioral data. Behavioral activity (seconds) was cumulated for the time frames provided by the PET data, and the differences between successive time points were used for correlation analysis. All scores indicate ranks, with a rank of 1 indicating the lowest score. Reproduced from Schulz et al. (2011).

in the detection of motivationally significant stimuli and during learning (Aosaki et al., 1994; Ravel et al., 2003).

The D2R occurs in two splice variants, termed D2-short (D2-S) and D2-long (D2-L). Ultrastructural examinations revealed that within the striatum the short variant (D2-S) is located mainly on axons of DA neurons, suggesting that D2-S is the autoreceptor, whereas the long variant (D2-L) is present on MSNs and on cholinergic interneurons (Khan et al., 1998). Spontaneous locomotor activity is altered in mice with deletions of both isoforms, but not in D2-L knockout mice, indicating that spontaneous behavioral activation is mediated by D2auto (Baik et al., 1995; Usiello et al., 2000).

Competition of ^{11}C-raclopride with phasic and tonic DA

^{11}C-raclopride binds specifically to D2R in the striatum. It is commonly assumed that the competition between ^{11}C-raclopride and DA for the same binding sites takes place in the synapse at postsynaptic D2Rs that are located on GABA-ergic D2 MSNs because under normal conditions, high-efficiency DATs remove DA from the synapse before it can escape to the extracellular space. Now it appears that this may not be the case. In the striatum, DATs are not located in the immediate vicinity of release sites, the active zone, but instead in axons and axon terminals outside the active zone (Nirenberg et al., 1996; Hersch et al., 1997). This suggests that DA is capable of diffusing from the release site into the extracellular space, consistent with the concept of volume transmission in which intercellular signaling transcends the boundaries of the synapse (Hersch et al., 1997; Gonon et al., 2000; Cragg and Rice, 2004; Sulzer et al., 2010). Moreover, most postsynaptic D2Rs are located away from the synapse (Hersch et al., 1995; Yung et al., 1995) so that DA must diffuse into the extracellular space to reach the postsynaptic targets (Gonon et al., 2000).

Learning and reward signals require brief pulses of DA or burst firing for the mediation of time-specific information (Schultz, 1998; Waelti et al., 2001). Such signals result in high (micromolar), phasic levels of DA capable of activating low-affinity DA receptors (May et al., 1988; Grace, 1991). Behaviorally relevant phasic events occur on a timescale of several hundred milliseconds as suggested by *in vivo* electrophysiological data (Schultz, 2007). Under normal conditions, phasic DA is rapidly cleared from the extracellular space by reuptake (Venton et al., 2003; Cragg and Rice, 2004). The half-life for uptake in the striatum was found to be in the range of 20–60 ms (Garris and Wightman, 1994; Gonon et al., 2000).

The slower tonic DA signaling refers to the spontaneous firing of neurons in the slow, single-spike mode, which is associated with tonic levels of DA in the extracellular space (Grace and Bunney, 1984; Grace, 1991). Tonic levels of DA reflect a steady-state baseline concentration of approximately 10–30 nM (Church et al., 1987; Venton et al., 2003). These levels can increase, such as during electrical stimulation of DA input pathways or after reuptake inhibition and can decrease, for example, after lesions of the mesolimbic input pathway (Gonon, 1988; Venton et al., 2003). Tonic DA is also regulated by glutamatergic input to DA terminals in the striatum, which is independent of impulse activity of DA neurons (Glowinski et al., 1988; Grace, 1991; Chéramy et al., 1998; Kulagina et al., 2001; Floresco et al., 2003; Goto et al., 2007). Tonic levels of DA are sufficient to activate high-affinity D2auto and are thus capable of modulating phasic events through effects on DA synthesis and release (Grace, 1991). The time course of tonic signaling is thought to range from seconds to minutes and perhaps hours (Grace, 1991; Suaud-Chagny et al., 1992; Zoli et al., 1998; Schultz, 2007). The long-lasting changes in extracellular levels of DA as measured with microdialysis might reflect slower behavioral processes, such as changes in behavioral excitation or satiation (Schultz, 2007). The sensitivity of PET data to DA in the nanomolar range and the relatively slow time course of the PET imaging technique (see below) suggest that under normal conditions the PET data in awake, behaving rats may be sensitive to changes in tonic levels of DA and in receptor occupancy of D2auto.

Temporal resolution of PET

The correlation of behavioral measures with the PET data necessitates the consideration of radiotracer kinetics. After a change in receptor occupancy by the endogenous ligand, it takes time for the concomitant changes in tracer binding to become measurable and for tracer levels to reach a new equilibrium. This timing is also dependent on the properties of the particular tracer used. The formalism for understanding the kinetics of competition between DA and ^{11}C-raclopride for the same receptor has been developed and validated in the rhesus monkey (Endres et al., 1997). Methods to optimize the approach were later developed (Watabe et al., 2000). Endres et al. (1997) show that a change in tracer binding after an acute challenge with amphetamine during a B-I study is clearly evident within 10 min, which is consistent with our data in the awake, behaving rat after a challenge with unlabeled raclopride (Schulz et al., 2011). We note that equilibrium is typically considered a requirement for a quantitative measurement of BP, but even though equilibrium may not be reestablished on the shortest behavioral timescales, our results suggest that relative changes in tracer binding do reflect relative changes in behavior in a fast if not fully quantitative way. These considerations imply that the correlations we have observed, which assume synchronous PET and behavioral data, might be improved by introducing a delay between the PET and behavioral data sets.

Acknowledgments

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