

Neonatal Hippocampal Damage Alters Electrophysiological Properties of Prefrontal Cortical Neurons in Adult Rats

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A neonatal excitotoxic lesion of the ventral hippocampus in the rat produces a variety of behavioral and cellular changes that remain latent until early adulthood. These delayed effects resemble many phenomena observed in schizophrenia, a neuropsychiatric disorder of early adult onset in which abnormal development of the hippocampus and prefrontal cortex has been postulated. Here we investigated the impact of this neonatal hippocampal lesion on the response of medial prefrontal cortical pyramidal neurons to specific afferent stimulation. Neonatal hippocampal damage altered the physiological responses of these neurons to electrical stimulation of midbrain dopaminergic–GABAergic projections, but not thalamic glutamatergic afferents. The lesion resulted in excessive firing of pyramidal neurons in response to mesocortical stimulation and this effect was not observed before adulthood or after similar hippocampal damage produced in adult rats. These data show that neonatal damage to the ventral hippocampus changes, in a developmentally specific manner, the nature of prefrontal cortical neuron responses to activation of projections from the ventral tegmental area, an effect that may explain the adverse impact of stress in schizophrenia.

Introduction

A role of dopamine (DA) in the pathogenesis of schizophrenia has been postulated for years, based largely on the therapeutic effects of antidopaminergic drugs (Creese *et al.*, 1976; Seeman, 1987). However, the search for the neural substrates of this disorder most consistently has yielded findings of abnormalities in cortical regions, particularly the prefrontal cortex (PFC) and hippocampal formation (Harrison, 1999; Selemon and Goldman-Rakic, 1999). Even though the diagnostic symptoms of the illness do not appear in most cases before early adulthood, there is evidence that the cortical abnormalities are present from early in life (Arnold *et al.*, 1991), suggesting abnormal development of the hippocampus and prefrontal cortex (Weinberger, 1987; Waddington, 1993; Weinberger and Lipska, 1995). A neonatal excitotoxic hippocampal lesion has been used as an animal model to test the hypothesis that an early developmental insult involving brain systems implicated in schizophrenia may remain latent until early adulthood (Lipska *et al.*, 1993, 1995; Saunders *et al.*, 1998). It is conceivable that hippocampal lesion-induced behavioral changes consistent with DA alterations are secondary to abnormalities of the PFC, a brain region receiving a dense innervation from the ventral hippocampus (Jay and Witter, 1991) and projecting to the ventral tegmental area (VTA) (Sesack and Pickel, 1992). Indeed, recent evidence indicates that neonatal hippocampal damage may affect PFC neuronal integrity. For example, in adult animals with this neonatal lesion there is reduced dendritic length and spine density of prefrontal pyramidal neurons (Lipska *et al.*, 2001), downregulation of GAD67 (the enzyme for GABA synthesis) mRNA expression (Lipska and Weinberger, 2000) and reduced *N*-acetylaspartate (NAA) levels (Bertolino *et al.*, 1999) in the PFC. Interestingly,

these measures have also been found to be reduced in the PFC of schizophrenia patients (Akbarian *et al.*, 1995; Bertolino *et al.*, 2000; Glantz and Lewis, 2000). Similarly, in monkeys with neonatal medial temporal removals (but again not with analogous ablations rendered in adulthood), NAA concentrations are reduced in dorsolateral PFC (Bertolino *et al.*, 1997) and the response of PFC to local infusion of amphetamine is abnormal (Saunders *et al.*, 1998). Furthermore, removing presumably abnormal PFC neurons from neonatally lesioned rats in adulthood normalized some of these effects (Lipska *et al.*, 1998), indicating that dysfunctional PFC neurons may be the effectors of DA-linked behavioral abnormalities.

Pyramidal neurons in the PFC exhibit a bistable membrane potential. A very negative resting membrane potential (down state) is periodically interrupted by plateau depolarizations (up state). Up states are believed to be driven by excitatory inputs (Amzica and Steriade, 1995; Wilson and Kawaguchi, 1996) and can be modulated by activation of VTA afferents (Lewis and O'Donnell, 2000). Given the heavy hippocampal–PFC projection, it is conceivable that up states can be affected by a ventral hippocampal lesion. Also, the output of the hippocampus controls activity in the VTA (Floresco *et al.*, 2001). A developmental lesion of the hippocampus could affect PFC cell properties and the activity of mesocortical DA projections, possibly resulting in abnormal responses of PFC neurons to VTA stimulation. Here we have evaluated PFC neuronal function in the lesioned animal using electrophysiological techniques and focusing on the nature of responses to VTA and thalamic afferent stimulation.

Materials and Methods

Animals

Pregnant Sprague–Dawley rats were obtained at 18 days of gestation from Taconic Farms (Germantown, NY). At postnatal day 6 (PD6), male pups (14–19 g) were separated into two groups. They were either lesioned with ibotenic acid or received a sham injection of artificial cerebrospinal fluid (aCSF). Four to nine pups were used for every surgery. A group of 11 rats (290–440 g) also received a lesion as adults. All experimental protocols were performed according to the USPHS *Guide for the Care and Use of Laboratory Animals* and had been approved by Albany Medical College Institutional Animal Care and Use Committee.

Lesions

Pups were anesthetized with hypothermia by placing them in wet ice. They were secured to a platform on a stereotaxic apparatus (D. Kopf, Tujunga, CA) and an incision was made through the skin. A cannula was lowered into the ventral hippocampus (AP, –3.0 mm; ML, ± 3.5 mm; DV, –5.0 mm; all relative to bregma) and 0.3 µl of ibotenic acid in aCSF (10 µg/µl) were delivered by a minipump at a rate of 0.15 µl/min. The procedure was repeated in the contralateral hippocampus. Sham-operated animals received 0.3 µl of aCSF on each side. The cannula was left in place for an additional 3 min. Animals were warmed up and returned to their cages, where they remained undisturbed until weaning.

A group of adult animals received similar lesions. These rats were anesthetized with equithesin (3 ml/kg, i.p.) and placed in a stereotaxic apparatus (D. Kopf). A cannula placed into the ventral hippocampus (AP, -4.4 mm; ML, \pm 5.0 mm; DV, -8.0 and -6.0 mm; all relative to bregma) was used to deliver 0.6 μ l of ibotenic acid (10 μ g/ μ l) or 0.6 μ l of vehicle, delivered at a rate of 0.2 μ l/min. Upon recovery from anesthesia, the animals were returned to their cages and recording sessions were conducted 1–3 weeks later.

Recording

A subset of animals was tested before puberty (PD 28–35) and others were tested as adults (PD56 and older). Recording procedures were as described elsewhere (Lewis and O'Donnell, 2000). Briefly, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) before being placed on a stereotaxic apparatus (D. Kopf). Recording and stimulating electrodes were lowered in the medial PFC (infralimbic and prelimbic areas), MD thalamus and VTA. Recording electrodes were glass micropipettes pulled with a Flaming-Brown puller (Sutter P-97) and filled with 3 M potassium acetate with 2% neurobiotin. Electrical signals were acquired with an amplifier (Neurodata IR-283), digitized with an interface board (DAP 3215; Microstar) and fed into a computer. Baseline recording and responses to electrical stimulation of VTA and MD were recorded. Following completion of the procedures, neurobiotin was injected into the neuron by applying positive current pulses (1 nA, 200 ms at 2 Hz for 5 min). Following histochemical procedures, this allowed for identification of cell type and location. Only neurons recorded from animals with a bilateral lesion and with recording and stimulating electrodes located in the intended sites were included in the analysis.

Stimulation

Concentric bipolar electrodes (NEX-100; Rhodes Medical Instruments) with 0.5 mm between the tips were employed for electrical stimulation. Electrodes were placed in the VTA (5.8 mm caudal to bregma; 0.5 mm lateral; 8.3 mm from skull surface) and the MD thalamic nucleus (2.8 mm caudal to bregma; 0.5 mm lateral; 5.3 mm from skull surface). All coordinates were taken from a stereotaxic atlas (Paxinos and Watson, 1998). Current pulses were generated by stimulus isolation units driven by a Master 8 Stimulator (AMPI, Jerusalem, Israel) controlled by a computer. Electrical stimulation of the VTA and MD was performed by delivering current pulses 0.5 ms in duration and 0.1–1 mA in amplitude every 10 s. The VTA was also stimulated with trains of five pulses at 20 Hz to mimic DA cell burst firing. This procedure was shown to evoke DA release in the nucleus accumbens (Gonon, 1997) and transitions to the up state in PFC neurons (Lewis and O'Donnell, 2000).

Lesion Analysis

The extent of damage was estimated roughly in all animals. Nissl staining was conducted in as many sections were necessary to include the entire rostrocaudal extent of the lesion. Areas with cell loss or cell disorganization were deemed as lesioned and lesion sizes were estimated roughly as the area at the coronal section in which they were largest, by measuring the diameter of damage extent. Since the lesion contours were irregular and in most cases difficult to determine precisely, there was some inaccuracy in the calculations. However, this was sufficient to separate animals with very discrete damage from animals with large lesions.

Results

In vivo intracellular recordings were performed from 61 neurons in layer V of the medial PFC of 47 rats. As reported earlier (Branchereau *et al.*, 1995; Lewis and O'Donnell, 2000), most PFC neurons (38/61) exhibited a bistable membrane potential characterized by a very negative resting potential (down state, -75.3 ± 8.2 mV; mean \pm SD) and depolarized plateaux (up state, -65.0 ± 7.6 mV) during which action potential firing could occur. These are similar to what has been reported in striatal (Wilson and Kawaguchi, 1996) and accumbens (O'Donnell and Grace, 1995) neurons, where the

'up' state depends on excitatory afferent inputs. Prefrontal 'up' events occurred at a frequency of 0.76 ± 0.29 Hz and lasted 382 ± 196 ms. The extent of damage in neonatally lesioned rats was variable; however, the results were not different when analyzing animals with small or large lesions separately. Therefore, the data were pooled. In the neonatally lesioned rats (Fig. 1), most neurons (17/23) exhibited a bistable membrane potential (Fig. 2A,B), a proportion similar to what has been observed in intact animals (Lewis and O'Donnell, 2000). In every case in which intracellular staining with neurobiotin was successful, the labeled cell exhibited the morphological characteristics of a pyramidal neuron (Fig. 2C). A bistable membrane potential was also observed in control groups, including sham animals and neonatal lesioned animals tested before puberty at PD28–35 (Fig. 3, Table 1). The frequency and duration of 'up' events, as well as other electrophysiological properties, were similar across these groups. Therefore, the excitatory inputs responsible for these state transitions do not appear to be affected by the neonatal hippocampal lesion. In the animals lesioned as adults, however, we could not find PFC pyramidal neurons with a bistable membrane potential (Table 1); these cells appeared to be deprived of transitions to the 'up' state and to be locked into the 'down' state. This suggests that hippocampal projections to the PFC may contribute to pyramidal cell 'up' state by providing sufficient glutamatergic activation to depolarize these cells. An adult lesion may reduce this drive, resulting in the loss of 'up' states. The sparing of 'up' states in PFC neurons from neonatally lesioned animals suggests that in the absence of functional hippocampal afferents during critical periods in development, other excitatory inputs may compensate for this deficit, restoring the levels of glutamate activation required for transitions to the 'up' state to occur.

To characterize the physiological responses of pyramidal neurons to afferent activation, we stimulated glutamatergic inputs from mediodorsal (MD) thalamus and dopaminergic-GABAergic inputs from the VTA. Stimulation of MD thalamic afferents evoked synaptic responses in all groups, which were similar to those observed in normal animals (Lewis and O'Donnell, 2000). These excitatory postsynaptic potentials (EPSP) were evoked in most neurons tested (13/17), including those recorded in neonatally lesioned animals (5/6). This suggests that the physiological effects of activating this thalamo-cortical projection are preserved in the presence of a neonatal hippocampal lesion.

Stimulation of the VTA, the source of DA innervation to the PFC, with trains of pulses mimicking DA cell burst firing resulted in a prolonged transition to the 'up' state in most neurons (Fig. 4), similar to a response dependent on D₁ receptor activation reported in intact animals (Lewis and O'Donnell, 2000). In normal animals, this prolonged 'up' state was typically accompanied by a suppression of PFC cell firing (Lewis and O'Donnell, 2000). Similarly, animals with the sham neonatal lesion tested in adulthood and animals with neonatal lesions tested before puberty revealed a VTA-evoked prolonged 'up' state with suppression in cell firing (Fig. 4). In animals with an adult ventral hippocampal lesion, VTA stimulation failed to evoke a prolonged 'up' state (Fig. 5). In animals lesioned as neonates and tested in adulthood, however, a prolonged 'up' state was evoked and it was accompanied by increased cell firing (6.5 ± 2.2 Hz, Table 1, Fig. 4). This was significantly different from all other groups (ANOVA, $F = 19.72$, $P < 0.00001$), which consistently exhibited firing suppression with VTA train stimulation (Table 1). There was no correlation between lesion size and the nature of the VTA-evoked responses. All groups

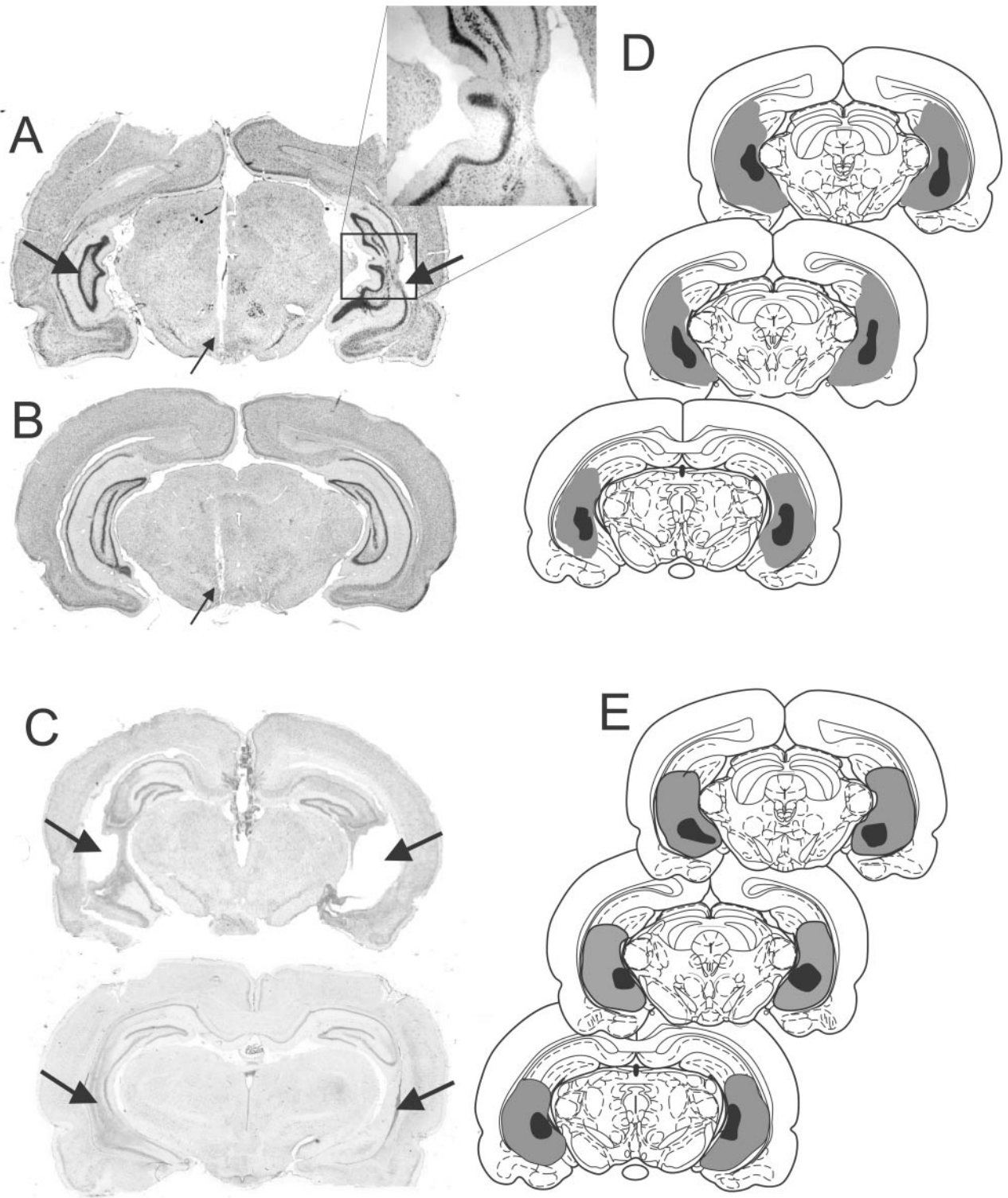


Figure 1. Ventral hippocampal lesion. (A) Microphotograph illustrating a typical ventral hippocampal lesion characterized by loss of neurons, gliosis and enlarged ventricles (arrows). Small arrows in this and the following panel indicate the location of tracks made by the VTA stimulating electrodes. Inset shows one side of lesion enlarged. (B) Brain from a sham animal showing an intact hippocampus. (C) Examples of a large (top) and a small (bottom) adult lesion. These lesions were characterized primarily by cell loss and cavitation rather than neuronal disarray (arrows). (D) Drawings illustrating the minimal (black) and maximal (gray) extent of hippocampal damage in neonatally lesioned animals. Coronal sections at 5.3, 5.8 and 6.0 caudal to bregma are displayed. (E) Similar drawings illustrating minimal and maximal extent of damage in adult-lesioned animals.

included animals with small and large lesions; animals with a neonatal lesion showed increased VTA-evoked firing regardless of lesion size and none of the other groups showed such effect,

regardless of lesion size. Thus, although VTA stimulation in neonatally lesioned animals elicited prolonged 'up' events in PFC neurons, these neurons appeared to be dysfunctional.

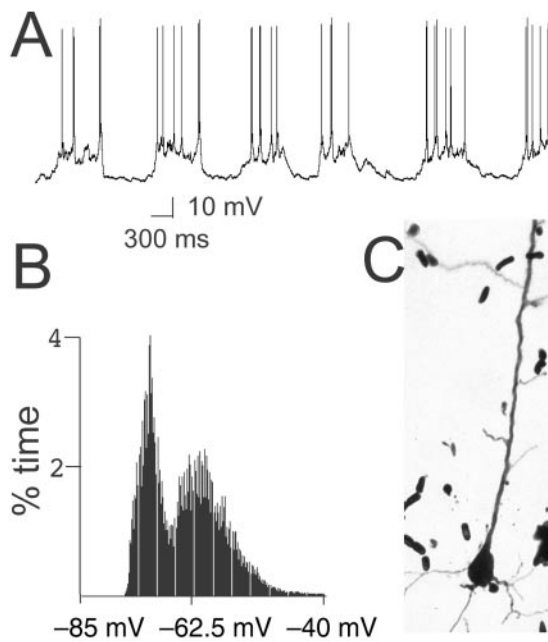


Figure 2. Most PFC neurons recorded in lesioned animals exhibited 'up' and 'down' membrane potential states. (A) Representative trace illustrating the spontaneous activity pattern in a neuron recorded from the medial PFC in this animal, exhibiting 'up' and 'down' membrane potential states. (B) Histogram plotting the proportion of different membrane potential values in the sample from which the tracing was extracted. A clear bimodal distribution is observed, with each mode corresponding to the respective membrane potential state. (C) Neurobiotin staining revealed this cell as a layer V pyramidal neuron, with an apical dendrite extending toward the pial surface (to the top of the figure).

Discussion

Neonatal hippocampal damage altered the response of PFC pyramidal neurons to VTA stimulation, but only after the animals reached adulthood. VTA stimulation evoked a dramatic increase in PFC action potential firing instead of the decrease in firing observed in all control groups and in untreated animals (Lewis and O'Donnell, 2000). Spontaneous membrane potential state transitions and the depolarization evoked by VTA stimulation were not affected by a neonatal hippocampal lesion. In animals lesioned as adults, however, we could not find neurons with 'up'-'down' membrane potential state transitions and VTA stimulation failed to evoke a depolarization.

In animals with a hippocampal lesion performed in adulthood, PFC pyramidal cell membrane potential was affected. 'Up' membrane potential states could not be detected. This indicates that hippocampal afferents may contribute significantly to the excitatory inputs that depolarize PFC neurons into the 'up' state. In the nucleus accumbens, a transection of the fimbria-fornix also resulted in the disappearance of spontaneous 'up' events (O'Donnell and Grace, 1995). In that study, electrical fornix stimulation evoked a transition to the 'up' state in accumbens neurons. More recently, we have shown that transitions to the 'up' state in PFC neurons could not be evoked by ventral hippocampal or fornix stimulation (Lewis and O'Donnell, 2000). Thus, although the hippocampal afferents may not be as important in driving 'up' states in PFC neurons as in accumbens neurons, their absence may impair synchronous excitatory activation, preventing spontaneous 'up' states in PFC cells. In animals with a neonatal lesion, on the other hand, the presence of spontaneous 'up' states suggests that, in the absence of

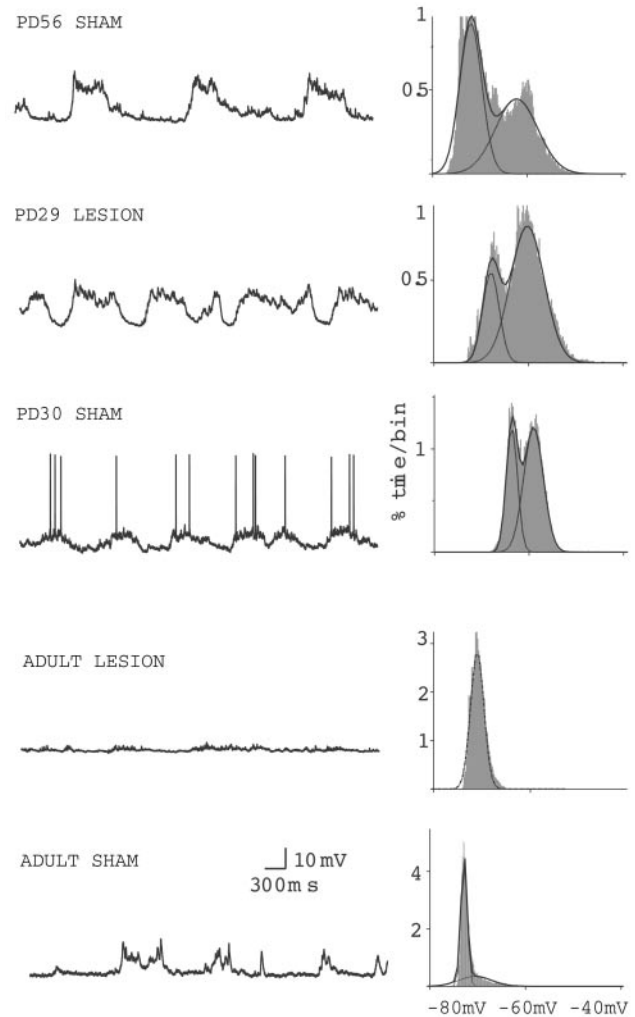


Figure 3. Spontaneous membrane potential fluctuation in all control groups. Representative traces from all groups are shown to the left. Histograms to the right reveal bimodal membrane potential distributions that can be fitted to a dual Gaussian function (dark lines). This was done in all neurons in all groups, except in animals lesioned as adults. In most neurons recorded in these animals, no up-down membrane potential state transitions could be observed (left) and the membrane potential distribution histogram could be fitted to a single, not dual, Gaussian function (right).

functional hippocampal innervation, other excitatory inputs may contribute to drive PFC 'up' states. Although speculative, it is probable that other cortico-cortical connections are recruited in this case.

In adult-lesioned rats, VTA stimulation did not evoke an increased firing rate as in neonatally lesioned rats and failed to evoke a membrane depolarization. This intriguing finding may indicate that VTA stimulation-dependent transitions to the 'up' state are indeed the result of enhanced glutamatergic activity, perhaps from hippocampal terminals. The difference in PFC response to VTA stimulation between neonatal and adult-lesioned animals indicates that this response is sensitive to developmental conditions.

In all other groups, VTA stimulation evoked prolonged depolarizations in PFC neurons, resembling the 'up' membrane potential state. This is similar to what we have observed in naïve animals (Lewis and O'Donnell, 2000). In that study, we demonstrated that this response could be reduced (but not blocked) by a D₁ antagonist. We interpreted such data as indi-

Table 1Electrophysiological properties of pyramidal PFC neurons in lesioned and control animals (all values mean \pm SD)

Lesion Tested at	PD6-lesion PD > 56	PD6-sham PD > 56	PD6-lesion PD28–35	PD6-sham PD28–35	adult-lesion PD > 56	adult-sham PD > 56
Bistable/total cells	11/16	9/10	6/7	8/12	0/7	4/9
Firing rate (Hz)	1.6 \pm 1.6	2.4 \pm 3.5	0.7 \pm 0.8	1.4 \pm 2.3	0.9 \pm 1.2	1.6 \pm 1.3
Membrane potential						
Down (mV)	-74.8 \pm 8.3	-79.3 \pm 7.7	-75.5 \pm 6.3	-71.3 \pm 7.8	-72.5 \pm 8.2	-71.0 \pm 6.6
Up (mV)	-62.7 \pm 5.6	-66.7 \pm 7.0	-65.2 \pm 6.9	-64.1 \pm 7.9	–	-60.8 \pm 4.6
Up frequency (Hz)	0.74 \pm 0.17	0.75 \pm 0.25	0.83 \pm 0.35	0.81 \pm 0.33	–	0.68 \pm 0.57
Up duration (ms)	443 \pm 223	383 \pm 249	307 \pm 101	394 \pm 175	–	305 \pm 146
Up state in response to VTA stimulation						
Duration (ms)	1273 \pm 539	1578 \pm 345	283 \pm 105	1029 \pm 697	–	1161 \pm 508
Firing rate ^a (Hz)	6.5 \pm 2.2	0	0	0	0	0
	(n = 5)	(n = 3)	(n = 4)	(n = 6)	(n = 4)	(n = 4)

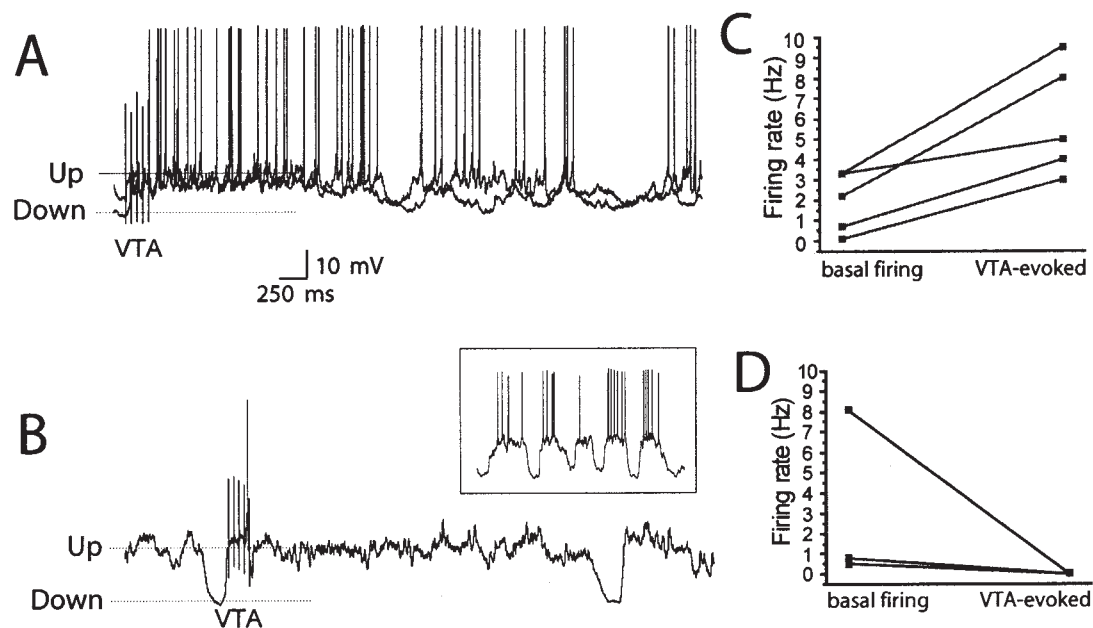
^aDuring the 1 s following the VTA train.

Figure 4. Burst stimulation of the VTA evoked a prolonged 'up' state, along with an increase in cell firing in neonatally lesioned animals tested as adults. (A) Overlay of two traces illustrating the depolarization in response to a 20 Hz train of five stimuli delivered to the VTA and the increased firing following stimulation. These traces were recorded from a medial PFC neuron in a rat lesioned at PD6 and tested as an adult. (B) Tracing from a sham-lesioned animal illustrating the prolonged 'up' state concomitant with a reduced firing that was characteristic of untreated rats. Inset: representative tracing from the same neuron, illustrating its baseline activity with action potential firing before VTA was stimulated. (C) Plot of spontaneous firing rate and VTA-evoked firing rate in all five neurons tested with VTA stimulation in this group, revealing an increase in firing rate by VTA stimulation. (D) Similar plot from all neurons in the neonatal sham group showing the normal decrease in firing by VTA stimulation.

cation of DA having a role in sustaining the depolarization to the 'up' state, an event dependent on synchronous excitatory inputs. The mechanisms involved in this action remain unclear. It is possible that VTA stimulation elicits a network change that enhances glutamatergic release causing a transition to the 'up' state in PFC pyramidal cells, with DA acting via D_1 receptors maintaining that 'up' state. Indeed, we have demonstrated recently that D_1 receptors can enhance NMDA-mediated responses in PFC pyramidal neurons recorded *in vitro* (Wang and O'Donnell, 2001). If there were such a role for DA in the PFC as to sustain 'up' states, one would expect 'up' events to occur synchronously with electrical activity in the VTA. We have preliminary evidence indicating that this is the case: simultaneous recordings from PFC pyramidal neurons and field

potentials in the VTA yield PFC 'up' states synchronous with VTA local field potentials (Peters and O'Donnell, 2000). Thus, in anesthetized animals electrical activity in the VTA can contribute to the periodic depolarizations that characterize the 'up' state in PFC pyramidal neurons.

In all control groups, the prolonged depolarization evoked by VTA stimulation was accompanied by a marked decrease in firing. This is also similar to what we observed in naïve animals with similar VTA stimulation (Lewis and O'Donnell, 2000). Chemical stimulation of the VTA had the same effect (Lewis and O'Donnell, 2000), indicating that the decrease in firing was dependent on activation of VTA projection neurons. A number of mechanisms can explain this finding. An early study (Bernardi *et al.*, 1982) reported that iontophoretic administration of DA in

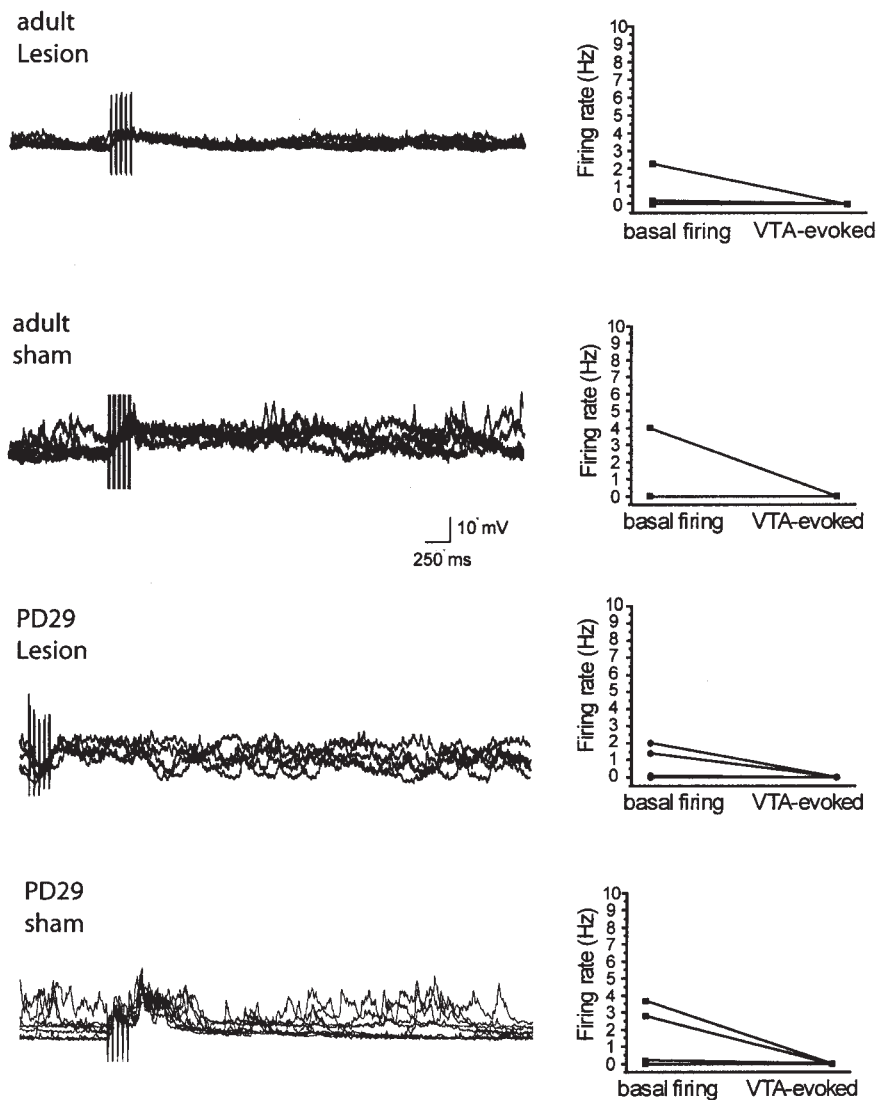


Figure 5. VTA-evoked responses. Traces from representative neurons (left) and plots of PFC cell firing rate before (basal) and following VTA stimulation. All traces are overlays of five records. Vertical lines indicate stimulus artefacts from VTA stimulation.

the PFC resulted in a depolarization concomitant with suppression in firing. This indicates that the suppression in firing may be an action dependent on DA in the PFC. DA released by VTA stimulation could act on pyramidal neurons or on GABAergic interneurons, resulting in interneuron-mediated inhibition of pyramidal cells. PFC GABA interneurons receive DA inputs (Sesack *et al.*, 1995) and possess DA receptors (Khan *et al.*, 2001). Another possibility would be that by stimulating the VTA we were activating GABA projection cells, which comprise a significant proportion of VTA neurons (Steffensen *et al.*, 1998; Carr and Sesack, 2000) and can inhibit PFC neurons (Pirrot *et al.*, 1992). The prolonged nature of this inhibition could be due activation of GABA-B receptors or to recruitment of a network of cortical interneurons. Thus, we believe three factors could contribute to the suppression in firing: activation of GABA projection neurons from the VTA; a DA effect on PFC GABA interneurons; and/or a direct action of DA on pyramidal neurons.

In animals with a neonatal hippocampal lesion, VTA stimulation evoked a dramatic increase in PFC cell firing. Although speculative, it is possible that any of the mechanisms mentioned

above could become altered in animals that developed with an abnormal ventral hippocampus. There is evidence, for example, of altered GABA interneurons in this model (Lipska and Weinberger, 2000). Given that the VTA and PFC are reciprocally connected (Thierry *et al.*, 1979; Au-Young *et al.*, 1999), electrical stimulation of the VTA could evoke antidromic firing in the PFC. However, it is unlikely that the increased PFC firing in response to VTA stimulation observed in animals with a hippocampal lesion is due to antidromic activation, because it is only observed in neonatally lesioned animals. One could argue that the hippocampal lesion would predispose to spread of antidromic activation. If this were the case, the adult lesion should also show an increased firing. Furthermore, antidromic responses typically involve a constant, short latency and are able to follow high-frequency stimulation (Seitun *et al.*, 1979). As illustrated in Figure 4A, no antidromic spikes were observed following the stimuli in the train. Therefore, we are confident that the increased firing observed in these animals is a synaptically mediated event. The source of such activation remains to be unveiled. The presence of a prolonged VTA-evoked

depolarization in these animals, an effect dependent on D₁ receptors (Lewis and O'Donnell, 2000), indicates that this lesion may not affect PFC D₁ receptors. It is possible that changes in D₂ receptors or GABA interneurons are responsible for the increase in firing. In summary, the basis for the delayed emergence of this electrophysiological abnormality may involve protracted postnatal developmental changes in PFC cytoarchitecture and DA innervation (Lambe *et al.*, 2000).

The altered response to VTA stimulation was present in all animals with neonatal ventral hippocampal damage, independently of lesion size. This observation was made in animals with a lesion encompassing most of the ventral and medial hippocampus, as well as in those with minimal lesion circumscribed to the ventral subiculum. Although this may seem surprising, there is a recent indication that a very subtle alteration in hippocampal activity (without lesion) by injection of tetrodotoxin (TTX) in neonates results in behavioral anomalies similar to those caused by the lesion (Lipska *et al.*, 2002). Thus, either a small lesion or a transient inactivation in this critical developmental period may result in changes in adult, but not prepubertal animals.

The increased firing during VTA-evoked 'up' events observed in neonatally lesioned animals may result in a loss of the filtering function DA exerts within this circuit (O'Donnell *et al.*, 1999). The effect of VTA stimulation in normal animals – i.e. a depolarization concomitant with a decrease in firing (Lewis and O'Donnell, 2000) – may provide a mechanism that brings PFC pyramidal neurons to a ready-to-fire state upon phasic activation of their DA afferents, but decreases baseline (or irrelevant) firing. Such a mechanism may contribute to the proposed role of DA systems in attributing salience to stimuli (Solomon and Staton, 1982; Schultz *et al.*, 1993). An increased firing evoked by VTA stimulation when the firing should have been reduced may result in inappropriate activation of PFC neurons. This could explain unusual responses of PFC to amphetamine in monkeys with neonatal medial temporal lobe lesions (Saunders *et al.*, 1998). In normal monkeys, caudate DA release was down-regulated after intra-PFC amphetamine injection, consistent with the electrophysiological data that after DA receptor activation in the PFC, pyramidal cell firing is reduced and, in turn, excitatory drive from PFC to brainstem DA neurons should be diminished. In contrast, the neonatally lesioned monkeys exhibited an increase in caudate DA release under these conditions. Our current data would predict precisely this result, as DA cell firing appears to be controlled by PFC inputs (Taber *et al.*, 1995) and a disrupted PFC may in turn alter DA systems projecting to the caudate (Bertolino *et al.*, 2000). Thus, animals that develop without proper hippocampal input exhibit disorganized PFC firing in response to DA. This may lead to recruitment of DA cells into excessive burst firing, and a vicious circle of cortical dysfunction and DA dysregulation that may characterize psychosis (Laruelle, 2000). In conclusion, this is the first direct evidence for a delayed physiological alteration in the VTA–PFC system following a neonatal hippocampal lesion.

Notes

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References

- Akbadian S, Huntsman M, Kim J, Tafazzoli A, Potkin S, Bunney W, Jones E (1995) GABA-A receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. *Cereb Cortex* 5:550–560.
- Amzica F, Steriade M (1995) Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *J Neurosci* 15:4658–4677.
- Arnold SE, Hyman BT, Van Hoesen GW, Damasio AR (1991) Some cytoarchitectural abnormalities of the entorhinal cortex in schizophrenia. *Arch Gen Psychiatry* 48:625–632.
- Au-Young SM, Shen H, Yang CR (1999) Medial prefrontal cortical output neurons to the ventral tegmental area (VTA) and their responses to burst-patterned stimulation of the VTA: neuroanatomical and *in vivo* electrophysiological analyses. *Synapse* 34:245–255.
- Bernardi G, Cherubini E, Marciani MG, Mercuri N, Stanzione P (1982) Responses of intracellularly recorded cortical neurons to the iontophoretic application of dopamine. *Brain Res* 245:268–274.
- Bertolino A, Saunders RC, Mattay VS, Bachevalier J, Frank JA, Weinberger DR (1997) Altered development of prefrontal neurons in rhesus monkeys with neonatal mesial temporo-limbic lesions: a proton magnetic resonance spectroscopic imaging study. *Cereb Cortex* 7:740–748.
- Bertolino A, Roffman J, Lipska BK, Van Gelderen P, Olson A, Weinberger DR (1999) Postpubertal emergence of prefrontal neuronal deficits and altered dopaminergic behaviors in rats with neonatal hippocampal lesion. *Soc Neurosci Abstr* 25:1294.
- Bertolino A, Breier A, Callicott JH, Adler C, Mattay V, Shapiro M, Frank JA, Pickar D, Weinberger DR (2000) The relationship between dorsolateral prefrontal neuronal *N*-acetylaspartate and evoked release of striatal dopamine in schizophrenia. *Neuropsychopharmacology* 22:125–132.
- Branchereau P, Van Bockstaele EJ, Chan J, Pickel VM (1995) Ultrastructural characterization of neurons recorded intracellularly *in vivo* and injected with lucifer yellow: advantages of immunogold-silver vs. immunoperoxidase labeling. *Microsc Res Tech* 30:427–436.
- Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci* 20:3864–3873.
- Creese I, Burt DR, Snyder SH (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* 192:596–598.
- Floresco SB, Todd CL, Grace AA (2001) Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *J Neurosci* 21:4915–4922.
- Glantz LA, Lewis DA (2000) Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Arch Gen Psychiatry* 57:65–73.
- Gonon F (1997) Prolonged and extrasynaptic excitatory action of dopamine mediated by D₁ receptors in the rat striatum *in vivo*. *J Neurosci* 17:5972–5978.
- Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 122:593–624.
- Jay TM, Witter MP (1991) Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of *Phaseolus vulgaris*-leucoagglutinin. *J Comp Neurol* 313:5774–5786.
- Khan ZU, Koulen P, Rubinstein M, Grandy DK, Goldman-Rakic PS (2001) An astroglia-linked dopamine D₂-receptor action in prefrontal cortex. *Proc Natl Acad Sci USA* 98:1964–1969.
- Lambe E, Krimer L, Goldman-Rakic P (2000) Differential postnatal development of catecholamine and serotonin inputs to identified neurons in prefrontal cortex of rhesus monkey. *J Neurosci* 20:8780–8787.
- Laruelle M (2000) The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. *Brain Res Rev* 31:371–384.
- Lewis BL, O'Donnell P (2000) Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential 'up' states in pyramidal neurons via D₁ dopamine receptors. *Cereb Cortex* 10:1168–1175.
- Lipska B, al-Amin H, Weinberger D (1998) Excitotoxic lesions of the rat medial prefrontal cortex. Effects on abnormal behaviors associated with neonatal hippocampal damage. *Neuropsychopharmacology* 19:451–464.
- Lipska BK, Weinberger DR (2000) To model a psychiatric disorder in

- animals: schizophrenia as a reality test. *Neuropsychopharmacology* 23:223-239.
- Lipska BK, Jaskiw GE, Weinberger DR (1993) Postpubertal emergence of hyperresponsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia. *Neuropsychopharmacology* 90:67-75.
- Lipska BK, Swerdlow NR, Geyer MA, Jaskiw GE, Braff DL, Weinberger DR (1995) Neonatal excitotoxic hippocampal damage in rats cause post-pubertal changes in prepulse inhibition of startle and its disruption by apomorphine. *Psychopharmacology* 132:303-310.
- Lipska BK, Kolb B, Halim N, Weinberger DR (2001) Synaptic abnormalities in prefrontal cortex and nucleus accumbens of adult rats with neonatal hippocampal damage. *Schizophr Res* 49(Suppl.):47.
- Lipska BK, Halim ND, Segal PN, Weinberger DR (2002) Effects of reversible inactivation of the neonatal ventral hippocampus in the adult rat. *J Neurosci* 22:2835-2842.
- O'Donnell P, Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J Neurosci* 15:3622-3639.
- O'Donnell P, Greene J, Pabello N, Lewis BL, Grace AA (1999) Modulation of cell firing in the nucleus accumbens. *Ann NY Acad Sci* 877:157-175.
- Paxinos G, Watson C (1998) *The rat brain in stereotaxic coordinates*, 4th edn. San Diego, CA: Academic Press.
- Peters YM, O'Donnell P (2000) Membrane potential state transitions in prefrontal cortical pyramidal neurons correlate with VTA field potentials *in vivo*. *Soc Neurosci Abstr* 26:1711.
- Pirot S, Godbout R, Mantz J, Tassin JP, Glowinski J, Thierry AM (1992) Inhibitory effects of ventral tegmental area stimulation on the activity of prefrontal cortical neurons: evidence for the involvement of both dopaminergic and GABAergic components. *Neuroscience* 49:857-865.
- Saunders RC, Kolachana BS, Bachevalier J, Weinberger DR (1998) Neonatal lesions of the medial temporal lobe disrupt prefrontal cortical regulation of striatal dopamine. *Nature* 393:169-171.
- Schultz W, Apicella P, Ljungberg T (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci* 13:900-913.
- Seeman P (1987) Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse* 1:133-152.
- Seitun A, Favale E, Gandolfo C (1979) Ortho-antidromic latency fitting and identification of antidromically activated CNS long-axoned neurons. *Neurosci Lett* 14:213-218.
- Selemon LD, Goldman-Rakic PS (1999) The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biol Psychiatry* 45:17-25.
- Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* 320:145-160.
- Sesack SR, Bressler CN, Lewis DA (1995) Ultrastructural associations between dopamine terminals and local circuit neurons in the monkey prefrontal cortex: a study of calretinin-immunoreactive cells. *Neurosci Lett* 200:9-12.
- Solomon PR, Staton DM (1982) Differential effects of microinjections of d-amphetamine into the nucleus accumbens or the caudate-putamen on the rat's ability to ignore an irrelevant stimulus. *Biol Psychiatry* 17:743-745.
- Steffensen SC, Svingos AL, Pickel VM, Henriksen SJ (1998) Electrophysiological characterization of GABAergic neurons in the ventral tegmental area. *J Neurosci* 18:8003-8015.
- Taber MT, Das S, Fibiger HC (1995) Cortical regulation of subcortical dopamine release: mediation via the ventral tegmental area. *J Neurochem* 65:1407-1410.
- Thierry AM, Deniau JM, Feger J (1979) Effects of stimulation of the frontal cortex on identified output VMT cells in the rat. *Neurosci Lett* 15:102-107.
- Waddington JL (1993) Schizophrenia: developmental neuroscience and pathobiology. *Lancet* 341:531-536.
- Wang J, O'Donnell P (2001) D₁ dopamine receptors potentiate NMDA-mediated excitability increase in rat prefrontal cortical pyramidal neurons. *Cereb Cortex* 11:452-462.
- Weinberger DR (1987) Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* 44:660-669.
- Weinberger DR, Lipska BK (1995) Cortical maldevelopment, anti-psychotic drugs, and schizophrenia: a search for common ground. *Schizophr Res* 16:87-110.
- Wilson CJ, Kawaguchi Y (1996) The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *J Neurosci* 16:2397-2410.