

Social Isolation Rearing Affects Prefrontal Cortical Response to Ventral Tegmental Area Stimulation

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Background: *Animals reared in social isolation exhibit attentional deficits that parallel those found in schizophrenia patients. Such disturbances are frequently attributed to a dysfunction of the mesocortical system. Here we investigated whether electrophysiologic characteristics of prefrontal cortical pyramidal neurons or mesocortical responses were changed in isolated animals.*

Methods: *In vivo intracellular recordings were obtained from prefrontal cortical pyramidal neurons in animals raised in social isolation or in socialized control animals before and after ventral tegmental area stimulation mimicking dopamine cell burst firing.*

Results: *Prefrontal cortical pyramidal neurons recorded from isolated animals showed bimodal characteristics resembling those of their socialized littermates. Stimulation of the ventral tegmental area evoked plateau depolarizations in both groups, but this was accompanied by abnormal firing or a short hyperpolarization in most of the isolated animals.*

Conclusion: *These findings suggest that social isolation rearing may affect mesocortical information processing.*

Key Words: Dopamine, electrophysiology, prefrontal cortex, schizophrenia, social isolation, ventral tegmental area

Raising animals in social isolation causes a variety of behavioral and neurochemical anomalies. These include altered sensorimotor gating of the acoustic startle response (Braff and Geyer 1990; Geyer et al 1993; Wilkinson et al 1994), hyperlocomotion in novel environments (Del Arco 2004; Jones and Robbins 1992), abnormal dopamine (DA) levels in both the nucleus accumbens and prefrontal cortex (PFC; Fulford and Marsden 1998; Jones et al 1992), altered efficacy of dopamine agonists (Howes et al 2000; Phillips et al 1994; Smith et al 1997), and changes in the expression of behavioral sensitization to repeated amphetamine (Weiss et al 2001). Morphologic alterations have also been observed in the form of reduced dendritic spine density in both the PFC and hippocampus (Silva-Gomez et al 2003). Many of these changes resemble to some extent manifestations observed in schizophrenia (Braff et al 1978; Braff and Geyer 1990; Garey et al 1998; Glantz and Lewis 1998), giving some credence to claims this animal model could be mimicking environmentally induced changes inherent to this disorder.

PFC pyramidal neurons recorded *in vivo* exhibit a negative resting membrane potential (down state) that is periodically interrupted by depolarized plateaus (up state). DA can modify the membrane potential, firing rate and regularity of PFC bimodal states (Bernardi et al 1982; Lewis and O'Donnell 2001; Peters et al 2004). Here we studied whether PFC membrane potential oscillations and responses to ventral tegmental area (VTA) stimulation were altered in animals reared in social isolation.

Methods and Materials

Social Isolation Rearing

Pregnant female Sprague-Dawley rats were obtained from Charles River Laboratories, (Wilmington, Massachusetts) and arrived at our animal facility on gestational day 14. All procedures involving animals were carried out in accordance with the

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National Institute of Health *Guide for the Care and Use of Laboratory Animals* and in agreement with Albany Medical College Institutional Animal Care and Use Committee. Male pups remained with the mother until weaning at day 21. They were separated into two experimental groups: one consisting of rats housed one per cage (isolated animals) and the other being three rats per cage (socialized animals). All animals were housed in a single room within the animal facility and maintained under these conditions for at least 8 weeks before conducting intracellular recordings. A total of six litters were used, and all provided isolated and social animals.

Electrophysiology

Animals were anesthetized with 400 mg/kg chloral hydrate intraperitoneally (i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, California). For the remainder of the experiment, chloral hydrate (20–30 mg/kg/hour, i.p.) was continuously administered with the aid of a minipump (Bioanalytical Systems, West Lafayette, Indiana) to maintain stable anesthesia levels. A rectal probe attached to a heating pad (Fine Science Tools, Foster City, California) was used to assess and maintain body temperature at 37°C. Bupivacaine (.25%) was administered subcutaneously before skin incisions. A small burr hole was drilled into the skull for PFC electrode placement (2.7 mm rostral to bregma [A]; .8 mm lateral [L]; –3 to –6 mm from the brain surface [V]). Glass electrodes were pulled from 1-mm OD Omegadot borosilicate glass tubing (World Precision Instruments, Sarasota, Florida). Electrodes were filled with 2 M potassium acetate and 2% Neurobiotin and advanced into the PFC with the aid of a hydraulic manipulator (Trent Wells, Coulterville, California). The intracellular signal was amplified using an IR-283 Neurodata amplifier (Cygnus Technology, Delaware Water Gap, Pennsylvania). Only neurons exhibiting a resting membrane potential of –50 mV or more negative, action potential amplitude of at least 45 mV and a bimodal membrane potential (i.e., exhibiting up and down states) were included in this study. Neurons were identified as having a bimodal membrane potential if a histogram of membrane potential values revealed two peaks separated by at least 5 mV and this distribution was best fitted to a dual Gaussian function (Lewis and O'Donnell 2000; Peters et al 2004). A concentric bipolar stimulating electrode was positioned in the VTA (A: –5.8 mm; L: .5 mm; V: –8.3 mm).

Comparisons of PFC neurons between groups were based on membrane potential, input resistance, duration of up states, and regularity of intervals between onsets of up states. At the completion of a 5-min baseline recording, the VTA was electri-

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cally stimulated with 20-Hz trains of five pulses (.5 msec, 1 mA) delivered every 15 sec by stimulus isolation units driven by a Master 8 Stimulator (AMPI, Jerusalem, Israel). Any change in membrane potential of at least 5 mV and consistently observed with repeated stimulation was characterized as a response. At the end of the recording, Neurobiotin was ejected by the application of current pulses (1 nA, 200 msec at 2 Hz) for at least 10 min.

Histology

Following completion of the experiments, animals were given a lethal dose of sodium pentobarbital (100 mg/kg) and perfused with saline followed by 4% paraformaldehyde. All brains were removed from the skull and placed in 30% sucrose for cryoprotection. Serial 30–60 μm coronal sections were cut using a freezing microtome. Sections containing Neurobiotin were further processed for exact identification of neuron and electrode placement. These sections were placed in .4% Triton X-100 (Sigma) in phosphate buffered saline for 1–2 hours followed by a 2-hour incubation period with Vectastain Elite avidin-biotin complex reagent (Vector Laboratories, Burlingame, California). Subsequently, sections went through a series of rinses before they were reacted with 3,3'-diaminobenzidine (DAB) and urea-hydrogen peroxide (Sigma Fast DAB set). All other sections were mounted on gelatin-coated slides and Nissl-stained for identification of electrode tracks with an Olympus Optical CH30 microscope (Tokyo, Japan) using the atlas of Paxinos and Watson (1998) for reference.

Results

Electrophysiologic recordings were performed in isolated ($n = 7$) or socialized ($n = 9$) animals. Only PFC pyramidal neurons that displayed a bimodal membrane potential were included in this study. PFC neurons recorded from socialized animals ($n = 11$) exhibited a down state of -74.1 ± 7.1 mV (Mean \pm SD) and an up state of -60.5 ± 7.1 mV. Fluctuations in the membrane potential occurred at a frequency of $.9 \pm .3$ Hz and up states lasted 549 ± 219 msec (Figure 1A). Action potential firing was observed at 1.0 ± 1.2 Hz, and action potential amplitude was 53.6 ± 6.5 mV, measured from threshold. Input resistance was 49.4 ± 20.8 M Ω . All these characteristics are similar to what has been previously reported (Lewis and O'Donnell 2000).

Membrane potential properties of PFC pyramidal neurons recorded from isolated animals ($n = 8$) were not different. The down state was -75.6 ± 6.0 mV, and up state was -61.8 ± 5.5 mV. Bimodal oscillations occurred at a frequency of $1.0 \pm .4$ Hz, with the up state lasting 540 ± 219 msec. Action potential firing was observed at 2.7 ± 4.4 Hz, and action potential amplitude was 64.6 ± 7.5 mV. Input resistance of neurons recorded from socially isolated animals was 40.2 ± 11.7 M Ω . Statistical analyses did not reveal differences between socialized and isolated animals in any of these membrane properties.

To characterize PFC state transitions, the regularity of up states and up state intervals was determined. The coefficient of variance (Cv) for up state duration was calculated from consecutive events contained in 20-sec epochs of recording. The Cv of up state duration was $.51 \pm .15$ in socials and $.43 \pm .07$ in isolated animals. There was no difference between groups in up state variability ($p = .21$). The interval between up state onsets in socialized animals was 1189 ± 655 msec and the regularity of these intervals was $.45 \pm .10$. The interval between up states in isolates was 1021 ± 461 msec and the Cv was $.56 \pm .24$. None of

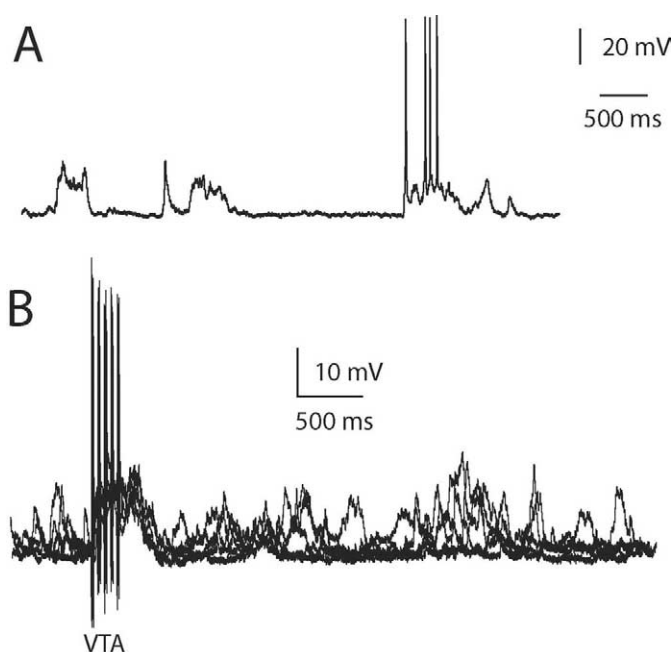


Figure 1. Electrophysiologic characteristics of prefrontal cortex pyramidal neurons from socialized animals. **(A)** Recording of spontaneous activity reveals slow oscillations in the membrane potential, alternating between negative down states and more depolarized up states. **(B)** Overlay of five repetitions of evoked responses to ventral tegmental area (VTA) stimulation with a burst of stimuli revealing a characteristic plateau depolarization.

these measures were different between isolated and socialized animals.

Following recording of spontaneous activity, the responses to VTA stimulation mimicking DA burst firing were measured. Stimulation of VTA evoked long-lasting depolarizations resembling up states in 9 of 10 neurons stimulated in socialized animals (Figure 1B). These plateau depolarizations lasted 944 ± 989 msec and were accompanied by firing suppression, as has been previously reported (Lewis and O'Donnell 2000). The remaining neuron did not respond to VTA train stimulation. On the other hand, only one of seven neurons stimulated in isolated animals exhibited this type of response. The remaining six neurons presented an early negative component before the plateau depolarization ($n = 2$; Figure 2), a depolarization with action potential firing ($n = 1$), or both ($n = 3$). Thus, the abnormal early inhibitory component was detected in five of seven neurons tested. The proportion of cells showing a normal response to VTA stimulation was lower in animals raised in social isolation ($p = .004$; Fisher Exact Test).

Discussion

In vivo intracellular recordings from isolated animals did not reveal any major differences in PFC pyramidal neuron membrane characteristics compared with socialized control animals. Up states were similar in duration, frequency, and membrane potential. Animals raised in social isolation exhibited abnormal responses to VTA stimulation, however. These results indicate that raising animals in social isolation may alter some specific neurophysiologic properties of the mesocortical system.

Isolation rearing has been proposed as a developmental animal model of schizophrenia. Both isolated animals and schizophrenia patients exhibit sensorimotor gating deficits, evi-

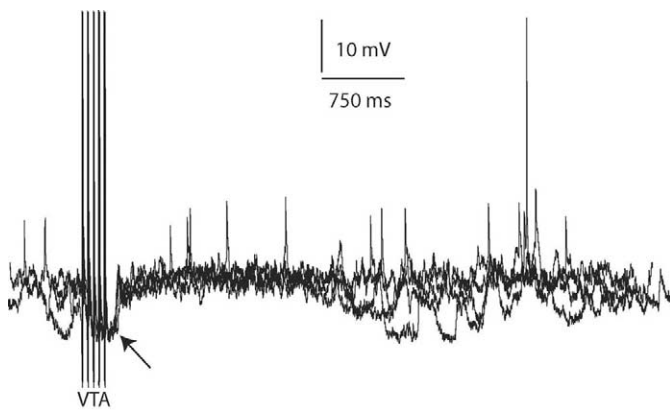


Figure 2. Abnormal response to ventral tegmental area (VTA) stimulation in animals raised in social isolation. Overlay of four traces of evoked response to VTA burst stimulation revealing a plateau depolarization preceded by a hyperpolarizing response (arrow).

denced by a disrupted prepulse inhibition (Braff and Geyer 1990; Geyer et al 1993) and a decreased PFC DA innervation has been observed in brains from isolated animals (Van den Berg et al 1999) and schizophrenia patients (Akil et al 1999). Other putative developmental animal models of this disorder also present sensorimotor gating impairment, as well as altered mesocortical function. For example, a neonatal ventral hippocampal lesion yields altered prepulse inhibition (Lipska et al 1995) as well as abnormal PFC responses to VTA stimulation (O'Donnell et al 2002). As in animals reared in isolation, the neonatal ventral hippocampal lesion failed to affect up–down oscillations in PFC neurons. The nature of altered mesocortical responses, however, was different here from what was observed in lesioned animals. Whereas the neonatal lesion yielded PFC neurons with increased firing in response to VTA stimulation (O'Donnell et al 2002), in many cases, isolation rearing resulted in an early inhibitory component in the response. The abnormal PFC firing following VTA stimulation in lesioned animals has been attributed to an altered DA innervation or lack of appropriate recruitment of cortical interneurons. The early hyperpolarization component following mesocortical stimulation in isolated animals may also suggest a disrupted balance of GABA and DA innervation of the PFC. Thus, isolation rearing can have a profound impact on the postnatal development of cortical DA receptors as evidenced by both abnormal responses to DA agonists (Leng et al 2004) and the reversal of isolation-induced sensorimotor deficits by antipsychotics (Bakshi et al 1998).

Social isolation rearing as an animal model mimics environmental factors such as stress, which may contribute to trigger the onset of schizophrenia and other psychiatric conditions in a predisposed brain (Benes 1997; Cannon et al 2003). A cohort of predisposing genes have been identified in the past few years (Harrison and Weinberger 2004), and most could be linked to developmental alterations in cortical regions (Corfas et al 2004; Raedler et al 1998; Waddington 1993). Mounting evidence now indicates that dopamine systems acquire their adult characteristics during puberty (Andersen et al 2001; Tarazi and Baldessarini 2000; Tseng and O'Donnell 2005). It is possible that the deleterious effects of social isolation on this important brain system, if acting on an already predisposed brain, could lead to expression of manifestations common to some psychiatric disorders.

This study provides the first evidence of neurophysiologic changes in PFC pyramidal neurons in animals raised in social

isolation. Dopamine inputs from the VTA are critical for proper information processing in the PFC. When the VTA is activated in response to salient stimuli, PFC responses may involve DA actions on both pyramidal cells and interneurons. The reduced pyramidal cell firing concomitant with the plateau depolarization observed in normal animals has been interpreted as necessary for filtering out weak or irrelevant stimuli (Lewis and O'Donnell 2000; O'Donnell 2003) and may be a critical PFC feature that allows response selection or goal-directed behavior. A disrupted cortical processing resulting from social isolation rearing may affect cortical functions dependent on responses to phasic VTA cell firing, altering basic mechanisms by which information is integrated, filtered, or both in the PFC. Thus, abnormal cortical patterns may emerge in isolates, causing sensorimotor gating deficits and abnormal behaviors that may resemble what is observed in schizophrenia and other psychiatric conditions.

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- Akil M, Pierri JN, Whitehead RE, Edgar CL, Mohila C, Sampson AR, Lewis DA (1999): Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. *Am J Psychiatry* 156:1580–1589.
- Andersen SL, LeBlanc CJ, Lyss PJ (2001): Maturation increases in c-fos expression in the ascending dopamine systems. *Synapse* 41:345–350.
- Bakshi VP, Swerdlow NR, Braff DL, Geyer MA (1998): Reversal of isolation rearing-induced deficits in prepulse inhibition by Seroquel and olanzapine. *Biol Psychiatry* 43:436–445.
- Benes FM (1997): The role of stress and dopamine-GABA interactions in the vulnerability for schizophrenia. *J Psychiatr Res* 31:257–275.
- 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.
- Braff DL, Callaway I, Geyer M, Glick I, Bali L (1978): Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychobiology* 4:339–343.
- Braff DL, Geyer MA (1990): Sensorimotor gating in schizophrenia: Human and animal model studies. *Arch Gen Psychiatry* 47:181–187.
- Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al (2003): Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions. *Schizophr Bull* 29:653–669.
- Corfas G, Roy K, Buxbaum JD (2004): Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci* 7:575–580.
- Del Arco A, Zhu S, Terasmaa A, Mohammed AH, Fuxe K (2004): Hyperactivity to novelty induced by social isolation is not correlated with changes in D2 receptor function and binding in striatum. *Psychopharmacol (Berl)* 171:148–155.
- Fulford AJ, Marsden CA (1998): Effect of isolation-rearing on conditioned dopamine release in vivo in the nucleus accumbens of the rat. *J Neurochem* 70:384–390.
- Garey LJ, Ong WY, Patel TS, Kanani M, Davis A, Mortimer AM, et al. (1998): Reduced dendritic spine density on cerebral cortical pyramidal neurons in schizophrenia. *J Neurol Neurosurg Psychiatry* 65:446–453.
- Geyer M, Wilkinson L, Humby T, Robbins T (1993): Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that of schizophrenia. *Biol Psychiatry* 34:361–372.
- Glantz LA, Lewis DA (1998): Dendritic spine density in schizophrenia and depression. *Arch Gen Psychiatry* 58:203.
- Harrison PJ, Weinberger DR (2004): Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Mol Psychiatry* (advance online publication July 20, 2004); doi:10.1038/sj.mp.4001558.
- Howes SR, Dalley JW, Morrison CH, Robbins TW, Everitt BJ (2000): Leftward shift in the acquisition of cocaine self-administration in isolation-reared

- rats: Relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. *Psychopharmacol (Berl)* 151:55–63.
- Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW (1992): Dopaminergic and serotonergic function following isolation rearing in rats: Study of behavioral responses and postmortem and in vivo neurochemistry. *Pharmacol Biochem Behav* 43:17–35.
- Jones GH, Robbins TW (1992): Differential effects of mesocortical, mesolimbic and mesostriatal dopamine depletion on spontaneous, conditioned, and drug-induced locomotor activity. *Pharmacol Biochem Behav* 43:887–895.
- Leng A, Feldon J, Ferger B (2004): Long-term social isolation and medial prefrontal cortex: Dopaminergic and cholinergic neurotransmission. *Pharmacol Biochem Behav* 77:371–379.
- Lewis B, O'Donnell P (2000): Ventral tegmental area afferents to the prefrontal cortex maintain membrane potential up state. *Cereb Cortex* 10:1168–1175.
- 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. *Psychopharmacol* 132:303–310.
- O'Donnell P (2003): Dopamine gating of forebrain neural ensembles. *Eur J Neurosci* 17:429–435.
- O'Donnell P, Lewis B, Lerman D, Weinberger DR, Lipska BK (2002): Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats. *Cereb Cortex* 12:975–982.
- Paxinos G, Watson C (1998): *The rat brain in stereotaxic coordinates*. San Diego, CA: Academic Press.
- Peters Y, Barnhardt NE, O'Donnell P (2004): Prefrontal cortical up states are synchronized with ventral tegmental area local field potentials. *Synapse* 52:143–152.
- Phillips GD, Howes SR, Whitelaw RB, Robbins TW, Everitt BJ (1994): Isolation rearing impairs the reinforcing efficacy of intravenous cocaine or intra-accumbens D1 and D2/D3 dopamine receptor agonists. *Psychopharmacol* 115:419–429.
- Raedler TJ, Knable MB, Weinberger DR (1998): Schizophrenia as a developmental disorder of the cerebral cortex. *Curr Opin Neurobiol* 8:157–161.
- Silva-Gomez AB, Rojas D, Juarez I, Flores G (2003): Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res* 983:128–136.
- Smith JK, Neill JC, Costall B (1997): Post-weaning housing conditions influence the behavioural effects of cocaine and d-amphetamine. *Psychopharmacol (Berl)* 131:23–33.
- Tarazi FI, Baldessarini RJ (2000): Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. *Int J Dev Neurosci* 18:29–37.
- Tseng KY, O'Donnell P (2005): Post-pubertal emergence of prefrontal cortical up states induced by D1-NMDA co-activation. *Cereb Cortex* 15:49–57.
- Van Den Berg CL, Van Ree JM, Spruijt BM (1999): Sequential analysis of juvenile isolation-induced decreased social behavior in the adult rat. *Physiol Behav* 67:483–488.
- Waddington JL (1993): Schizophrenia: Developmental neuroscience and pathobiology. *Lancet* 341:531–536.
- Weiss IC, Domeney AM, Heidbreder CA, Moreau JL, Feldon J (2001): Early social isolation, but not maternal separation, affects behavioral sensitization to amphetamine in male and female adult rats. *Pharmacol Biochem Behav* 70:397–409.
- Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW (1994): Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. *Neuropsychopharmacology* 10:61–72.